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STUDIES ON THE PHARMACOLOGICAL ACTION OF OXIDIZING SUBSTANCES[1]

A. S. LOEVENHART AND W. E. GROVE

From the Pharmacological Laboratory of the University of Wisconsin

Received for publication September 2, 1911

The only two fundamental processes occurring in living things of which any considerable amount of knowledge has been accumulated are processes of oxidation and hydrolysis. Under the term oxidation we include reduction because every oxidation implies a reduction and under the term hydrolysis is included the reverse process, namely, hydrosynthesis.

In spite of the fact that processes of oxidation play such an important rôle in the life of every living cell yet we have, at present, little knowledge of the effect of increased physiological oxidation on the functions of cells or tissues. The ability of oxygen to increase oxidation in higher animals is limited by the oxygen carrying power of the blood.

In arterial blood the haemoglobin is almost saturated with oxygen and the increase in the oxygen in the blood when the pure gas is breathed is very slight. The work with ozone and the alkalis has likewise not thrown very much light on the reaction of cells to increased oxidation.

Pharmacological studies of the action of many of the more violent and destructive oxidizing agents have been made. Most of these cannot be used intravenously. Many cause methaemoglobin formation and hydrogen peroxide when injected intravenously causes death by gas embolism. Many of these substances, such as the chlorates, are not reduced in their passage through the body, from which we may conclude that their oxygen is not

[1] The expense of this investigation has been partially defrayed by a grant from the Rockefeller Institute for Medical Research.

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available for physiological oxidation. Physiological oxidations are brought about through the agency of the oxidases. Kastle and Loevenhart[2] showed that the organic peroxides in general have many of the characteristics of certain of the oxidizing ferments and they were led to agree with Bach[3] that the production of organic peroxides in the cell would readily account for much that is known regarding vital oxidation. In 1905 Loevenhart[4] published a short paper on the therapeutic uses of benzoyl peroxide wherein it was shown that this substance is an ideal local antiseptic.

We were led to believe in the course of this work that were it possible to obtain a soluble preparation of the type of benzoyl peroxide it would prove of great value as an internal antiseptic which could be administered intravenously. In the second place, we were anxious to investigate the pharmacological action of some substance which could be injected intravenously and which, from its chemical nature, might be supposed to increase the normal physiological oxidation of cells.

Several substances are known which interfere with vital oxidation without apparently having any other effect, and cells may readily be subjected to conditions in which their processes of oxidation are inhibited. For these reasons we are well acquainted with the phenomena accompanying a decrease in the vital oxidation. On the other hand, it has thus far been impossible to study the effect of increased oxidation in living tissues and accordingly the investigation of the physiological response to increased oxidation is of the most vital importance to physiology and pharmacology.

It was in the hope of throwing light on these two propositions that we undertook the study of compounds containing oxygen in a form which might be available physiologically. Up to the present time we have investigated the action of only three such

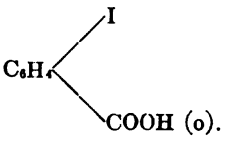
[2] Amer. Chem. Jour., 26, 539 (1901).

[3] Compt. rend., 124, 951 (1897).

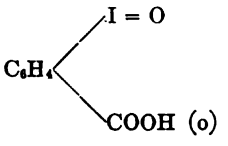
[4] Therapeutische Monatshefte., 19, 426, (1905). The views here expressed regarding the therapeutic value of this drug in the conditions mentioned have since been confirmed by a great many clinical observations in the hands of a number of men.

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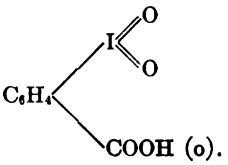
substances, namely, sodium iodosobenzoate, sodium iodoxybenzoate, and the sodium salt of phthalic peroxide. In the present communication we shall merely present the studies of the first two substances. Throughout the work we have studied the effect of sodium iodbenzoate, the mother substance as a control. The formulas of the three acids are as follows:[5]



Iodbenzoic acid



Iodosobenzoic acid



Iodoxybenzoic acid

All of these acids when perfectly pure are white or practically so. When nearly pure they have a faint cream color. Orthoiodbenzoic acid has been long known. Iodoso- and iodoxybenzoic acids were first prepared by Victor Meyer and his coworkers.[6] The free acids are insoluble in water but the sodium salts of all are very soluble. The principal interest connected with the compounds centers around the oxygen combined with the iodine. This oxygen is readily liberated in consequence of which iodoso- and iodoxybenzoic acids are capable of oxidizing many substances. Iodbenzoic acid of course has no oxidizing action. Iodoso- and iodoxybenzoic acids when reduced yield iodbenzoic acid. These two acids oxidize hydriodic acid quantitatively and the liberated iodine can be estimated readily by titration with $\frac{N}{10}$ sodium thiosulphate using starch paste as the indicator. This, together with the melting points, affords a ready method of determining the purity of these substances and all material used in our work was analysed in this way. The calculated amount of active oxygen contained in these substances is as follows:

	Per cent
Iodbenzoic acid	0.00
Iodosobenzoic acid	6.06
Iodoxybenzoic acid	11.43

[5] There are other views regarding the structure of iodosobenzoic acid but it is unnecessary to consider them in connection with our work.

[6] Meyer and Wachter: Berichte d. d. chem. Ges., 25, 2632 (1892). Askenasy and Meyer: Berichte d. d. chem. Ges., 26, 1354 (1893). Hartmann and Meyer: Berichte d. d. chem. Ges., 26, 1727 (1893); 27, 1600 (1894).

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It is interesting and important in the light of the pharmacological action of these substances to compare their strength as acids. Ortho-iodbenzoic acid is the strongest of the three iodbenzoic acids. Iodosobenzoic acid is extremely weak. Its salts are decomposed by carbon dioxide and the acid precipitated. Iodoxybenzoic acid has marked acid properties. Askenasy and Meyer[7] determined the dissociation constants of iod- and iodosobenzoic acids, which were found to be as follows:

Iodbenzoic acid	k. =0.132
Iodosobenzoic acid	k. =0.00006
Carbonic acid	k. =0.0013

The dissociation of iodoxybenzoic acid has apparently not been determined. In aqueous solution it has a very sour taste. It decomposes carbonates with liberation of carbon dioxide and on passing a current of carbon dioxide through a solution of barium iodoxybenzoate there is no precipitation of barium carbonate.

A word as to the methods which we have used in preparing these substances may not be amiss, although we have not departed very widely from the methods given by Meyer and his coworkers. We have compared the various methods which they used both as to the purity of the products and the yield.

Preparation of iodbenzoic acid: Suspend 20 grams anthranilic acid in water and add 50 cc. concentrated hydrochloric acid, warm to bring into solution, then cool to below 10°. Add 10 grams sodium nitrate in solution and after about five minutes add 30 grams of potassium iodide in solution. When the reaction is nearly over the contents of the flask are heated on the water bath. The buff colored precipitate of iodbenzoic acid is filtered off on a perforated plate with suction and dried. The yield is from 1¼ to 1½ times the weight of the anthranilic acid employed. This crude product is sufficiently pure to prepare the crude iodosobenzoic acid. For pharmacological work it can be purified in various ways; by dissolving in alkalis and precipitating with acid or by recrystallization or sublimation. In our hands the simplest way of procuring a perfectly pure white product in sufficient

[7] Berichte d. d. chem. Ges., 26, 1354 (1893).

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quantities is to transform it into pure iodosobenzoic acid and then reduce this to iodbenzoic acid. This yields a beautiful product.

Preparation of iodosobenzoic acid: Thirty grams of crude iodbenzoic acid, 700 cc. of 2.3 per cent potassium permanganate, 270 cc. 20 per cent sulphuric acid and 180 cc. of water were boiled together for fifteen minutes. Then boiling water was added until the total volume was 2 liters. The boiling was then continued for fifteen minutes, filtered through a hot water funnel and the iodosobenzoic acid which crystallized out on cooling was filtered off, dried, weighed and analysed. The yield is about gram for gram of the iodbenzoic acid used. The material contains from 5.88 per cent to 5.90 per cent of active oxygen. This crude product is sufficiently pure for the preparation of iodoxybenzoic acid. To obtain a pure product, the crude acid is dissolved in sodium hydroxide, a great excess being avoided, filtered and precipitated by passing a current of carbon dioxide into the solution. A second, and frequently a third precipitation of iodosobenzoic acid may be obtained by passing more CO_2 through the filtrates. A beautiful product is invariably obtained by this method. It is almost perfectly white. The crystals are small, due to the agitation of the solution. The yield is good, the loss being less than 10 per cent. This product invariably has a content of 6.03 to 6.06 per cent of active oxygen. This method of preparation is far superior to the first method proposed by Meyer and Wachter in which the iodbenzoic acid was oxidized by means of fuming nitric acid. This method of purification is the best we have been able to devise and leaves little to be desired.

Preparation of iodoxybenzoic acid: We have employed the chlorine method of Hartmann and Meyer. This consists in passing a current of chlorine through an alkaline solution of sodium iodosobenzoate, keeping the temperature down by immersing the bottle in cold water. Meyer and Hartmann give directions for separating the iod-, iodoso- and iodoxybenzoic acids which are the products of the reaction. We have found that crude iodosobenzoic acid can be employed and that by carefully controlling the temperature at which the chlorine acts, it is possible to obtain at once almost pure iodoxybenzoic acid. It may

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be rendered perfectly pure by a single recrystallization. The temperature at which the chlorine acts must be neither too high not too low. Considerable heat is generated in the process and if the reacting mixture becomes too warm the product will be worthless. If the mixture is kept too cold considerable quantities of iod- and iodosobenzoic acids will be found mixed with the iodoxybenzoic acid. We have obtained the best results by filtering the solution before passing the current of chlorine through it and by immersing the bottle in which the oxidation occurs in tap water at from 15° to 20° and towards the end of the process adding warm water to the bath to bring its temperature up to 30° or 35°. The yield of iodoxybenzoic acid is about 75 per cent of the crude iodosobenzoic used.

THE PHARMACOLOGICAL ACTION OF THE THREE ACIDS AND OF THEIR SODIUM SALTS

As far as we have been able to find the action of iodoxybenzoic acid on living organisms has not been studied. No particular attention has ever been paid to the action of iodbenzoic acid.

The only study of the action of iodosobenzoic acid which we have been able to find is that of R. Heinz.[8] He studied iodosobenzoic acid with the view of investigating the action of nascent iodine in the body, thinking that by giving potassium iodide by mouth and sodium iodosobenzoate locally, iodine would be liberated in the body in the nascent state. In speaking of sodium iodosobenzoate he says: "Aus diesem Grunde ist es für Thierversuche nicht verwendbar, indem es äusserst heftig reizt, das Blut rasch auflöst und bräunt, die Gewebe aufquellen und, soweit sie bluthaltig sind, missfarben macht." Heinz described his preparation of iodosobenzoic acid as consisting of yellowish leaflets. This would indicate that he used the fuming nitric acid method described by Meyer and Wachter, in preparing it.

He states that the sodium salt is strongly alkaline and for this reason is not available for animal experiments. In his experiments he therefore employed only the slightly soluble free iodo-

[8] Virchow's Archiv., 155, 44 (1899).

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sobenzoic acid in the form of a powder or emulsified with gum arabic or starch. Heinz found that iodosobenzoic acid when placed in the stomach of a rabbit causes hyperaemia and small haemorrhages of the gastric mucosa. This he attributes to its local irritating action, and states that the effect can also be shown by placing some of the powder in the eye (without simultaneously injecting sodium chloride). This irritating action he states is due in part to the acid nature of the substance and in part is characteristic of these compounds, all iodoso compounds even in neutral solution being more or less irritating.

Heinz also studied to some extent the fate of iodosobenzoic acid in the body. He showed that none escapes in the urine as iodosobenzoic acid nor as iodosohippuric acid, because the urine does not libertate iodine from hydriodic acid. Within the first hour, Heinz found that the urine gives a distinct test for iodine on ashing while the test for sodium iodide is negative. Later he found inorganic iodides present in the urine. He states, therefore, that in the body a gradual change in the iodosobenzoic acid occurs whereby iodine is liberated and combines with the alkali in the tissue juices to form iodides. This only occurs very slowly and produces no recognizable physiological effect.

The local action of the substances: Iodbenzoic acid dusted into the eye of a rabbit causes a slight degree of congestion after twenty-four hours, but within forty-eight hours the eye is perfectly normal. Sodium iodbenzoate when instilled into the conjunctival sac or injected subcutaneously in $\frac{N}{80}$ solution is without irritating action.

Iodosobenzoic acid: Heinz found the free acid as well as the sodium salt extremely irritating. He describes the material used as having a light yellow color, whereas the pure substance is almost white. Hence we determined to study the local action of the pure substance. When the dry white powder is dusted into one eye of the rabbit (the other eye being kept as a control), the reaction is practically the same as that resulting from any bland insoluble powder. There is no redness nor injection of the vessels. The eye was not kept closed, there was no increase in the secretion, no extension of the nictitating

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membrane, no change in the size of the pupil. After instilling two drops of a $\frac{N}{20}$ solution of sodium iodosobenzoate into the conjunctival sac of a young dog four times within a period of eight minutes, the eye did not show the slightest reaction and the animal apparently suffered no discomfort in any way. One of us (Loevenhart) took by mouth 0.3 gram of free iodosobenzoic acid suspended in water and three hours later 0.5 gram and $2\frac{1}{2}$ hours later 0.5 gram, a total of 1.3 grams within $5\frac{1}{2}$ hours without the slightest symptom being noted. A number of observations were made on the effect of sodium iodosobenzoate when injected subcutaneously and intraperitoneally.

A description of one experiment with the findings will suffice to illustrate its action on subcutaneous tissue.

Two cubic centimeters of a sterile $\frac{N}{30}$ (0.953 per cent) solution of sodium iodosobenzoate were injected subcutaneously on the back of a rabbit near the middle line about 6 cm. above tail. After forty-eight hours the animal was killed by a blow on the neck and examined.

Autopsy notes: There is a thickening of the subcutaneous tissues at the site of the injection which has a semifluid feel. On incision there is found a wet gelatinous infiltration of the subcutaneous tissues, extending from 2 to 3 cm. above the puncture wound around the animal nearly to the midline on the abdomen, down both thighs a considerable distance and posteriorly to a point 1 to 2 cm. below the tail. There is marked injection of the smaller vessels of the fascia and muscles, minute haemorrhages in the fascia and one quite large haemorrhage in the muscle. There is one opaque, apparently necrotic area, in the lumbar muscles below the area of the haemorrhage.

Local action in the peritoneal cavity: Fifteen cubic centimeters of various strengths of sodium iodosobenzoate from $\frac{N}{80}$ to $\frac{N}{100}$ were placed in the peritoneal cavity of rabbits aseptically. Under anaesthesia a very short incision was made in the abdominal wall down to the peritoneum. The rounded end of a fine pipette was thrust through the peritoneum and the solution allowed to flow in. No stitch was taken in the peritoneum. The abdominal muscle and skin were sewed up separately. Three days later the animals

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were killed by a blow. When 15 cc. of $\frac{N}{100}$ solution (0.286 per cent) of the sodium salt were used there were no symptoms whatever. At autopsy it was found that the peritoneal surfaces were glistening and covered between the coils of intestine by a small amount of blood stained serous fluid. Beneath the abdominal wound and adherent to the parietes there was a mass of blood stained fibrin. The omentum showed minute haemorrhages and there was a similar fibrinous mass adherent to it. There were scattered minute subserous haemorrhages over the intestines and the parietal peritoneum in the pelvic region. With stronger solutions the irritating action of the substance is much more marked and may prove fatal.

Iodoxybenzoic acid: On account of its markedly acid properties, free iodoxybenzoic acid is more irritating than free iodosobenzoic acid, but the sodium salt of iodoxybenzoic acid is in comparison to sodium iodosobenzoate a bland substance. One cubic centimeter of $\frac{N}{80}$ sodium iodoxybenzoate was injected subcutaneously in a rabbit and 8 cc. of $\frac{N}{80}$ solution were placed in the peritoneal cavity. There were no symptoms. Forty-eight hours later the rabbit was killed by a blow on the neck. The site of the subcutaneous injection was dry and showed no sign of haemorrhage or filtration. The abdominal wound was clean and showed a few minute subcutaneous haemorrhages in the immediate vicinity. There was a small collection of fibrin on the peritoneal surface of the wound and one or two flakes of fibrin on the surface of the intestine. The peritoneal surfaces were moist and glistening and there was no excess of fluid in the peritoneal cavity, which was perfectly normal except for one minute haemorrhage in the omentum. The lungs, heart and all the organs were perfectly normal.

We may summarize the local action of the three acids and their sodium salts as follows: Iodbenzoic acid is slightly irritating in consequence of its acid character. Free iodosobenzoic acid when pure is a very bland substance. When dusted into the eye or when taken into the stomach it produces no reaction. Iodoxybenzoic acid is quite irritating in consequence of its acid properties. Sodium iodbenzoate and iodoxybenzoate are quite bland. Sodium iodosobenzoate is exceedingly irritating especially to the subcutaneous tissue and the serous surfaces.

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A comparison of taste of iodbenzoic, iodosobenzoic and iodoxybenzoic, as well as that of their sodium salts is quite interesting. Iodbenzoic acid has a very faint sour taste when the dry powder is placed on the tongue. Iodosobenzoic acid has the peculiar taste of hydrogen peroxide. In larger quantities it causes a peculiar peppery sensation.

Iodoxybenzoic acid has an intensely sour taste as one should expect from its strongly marked acid properties. It also has a metallic taste and causes the peppery sensation noted in the case of iodosobenzoic acid. In comparing the tastes of the sodium salts of the three acids $\frac{N}{100}$ solutions were employed. Several persons tasted the solutions for us without having any knowledge of what was expected of them. Of the three salts that of iodosobenzoic acid has by far the strongest taste and its taste is almost identical with that of hydrogen peroxide, so much so in fact, that one is surprised to find that the saliva is not frothy as occurs when hydrogen peroxide is taken into the mouth. Pure sodium iodbenzoate is practically tasteless in $\frac{N}{100}$ solution. Sodium iodoxybenzoate also tastes very much like hydrogen peroxide solutions but the taste is not nearly as strong as that of sodium iodosobenzoate solutions of corresponding strength.

THE PHYSIOLOGICAL AVAILABILITY OF THE OXYGEN COMBINED WITH THE IODINE IN IODOSOBENZOIC AND IODOXYBENZOIC ACIDS

As already pointed out the oxygen combined with the iodine in these compounds is very active. It is capable of quantitatively oxidizing hydriodic acid with the liberation of iodine instantly at room temperature. Nevertheless the ordinary chemical activity of the oxygen in any compound does not necessarily mean that such oxygen will be available physiologically. The sodium salts of both acids immediately oxidize haemoglobin to oxyhaemoglobin.[9] A dilute solution of oxyhaemoglobin obtained by

[9] It should be pointed out that on treating the sodium salts of iodoso and iodoxybenzoic acids with blood there is no liberation of gaseous oxygen as in the case of hydrogen peroxide.

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laking blood with distilled water was treated with Stokes reagent until the two absorption bands of oxyhaemoglobin fused into the single band of haemoglobin and the color of the blood indicated the change from oxyhaemoglobin to haemoglobin. On adding the $\frac{N}{80}$ solution of the sodium salt of either iodoso- or iodoxybenzoic acid the two bands of oxyhaemoglobin immediately appeared and the color changed back to the bright red of an oxyhaemoglobin solution.

This very simple experiment proved that the oxygen which these substances contain is available physiologically through the intermediation of haemoglobin, but we were also anxious to determine whether or not the tissues themselves might not be able to utilize the oxygen combined with the iodine in these substances in much the same manner that they utilize the molecular oxygen, which in the higher animals is derived from the oxyhaemoglobin. So far as we know no attempt has been made to supply oxygen to the tissues in any other form than as molecular oxygen.[10]

In order to form some idea of the direct physiological availability of the active oxygen contained in our substances we have made use of the various oxidase and peroxidase reactions.

Neither the free acids nor the sodium salts are capable of oxidizing either guaiacum or aloin in the presence or absence of blood or tissue extracts so that as far as these substances are conserved our results are purely negative. Positive results were obtained, however, when we employed the sodium salt of phenolphthalin, the oxidase and peroxidase reagent introduced by Kastle and Shedd.[11] This substance was used by Kastle[12] in his extensive studies on the peroxidase of blood. Sodium iodosobenzoate is incapable of oxidizing phenolphthalin to phenolphthalein under

[10] By this statement we mean any modern attempt by experimental methods. The old idea that the chlorates given by mouth supply the body with oxygen is only of historical interest as showing how completely the application of ordinary test tube chemistry fails when applied to living things without experimental basis. This is perhaps more true in oxidative reactions than in reactions of any other type.

[11] Amer. Chem. Jour., 26, 526 (1901).

[12] "Variations in the peroxidase activity of the blood in health and disease." Washington, D. C., Hygienic Laboratory Bulletin, No. 31, 1906.

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the conditions of our experiments, but in the presence of diluted blood it effects this oxidation, thus indicating that the oxygen contained in sodium iodosobenzoate is capable of being activated by the peroxidase of the blood in this reaction and supporting the view that the active oxygen which it contains is probably capable of being utilized directly by the tissue in certain oxidations. The following may be given a typical experiment: Mixtures of diluted human blood (diluted approximately 1 : 1000) $\frac{N}{100}$ sodium phenolphthalin, $\frac{N}{100}$ sodium iodosobenzoate and water were placed in test tubes. The total volume was in each case made up to 7 cc.

(1) 0.2 cc. phenolphthalin
6.8 cc. water

(2) 0.2 cc. phenolphthalin
0.2 cc. diluted blood
6.6 cc. water

(3) 0.2 cc. phenolphthalin
0.2 cc. iodosobenzoate
6.6 cc. water

(4) 0.2 cc. phenolphthalin
0.2 cc. iodosobenzoate
0.2 cc. diluted blood
6.4 cc. water

(5) 0.2 cc. diluted blood
6.8 cc. water

(6) 0.2 cc. diluted blood
0.2 cc. sodium iodosobenzoate
6.6 cc. water.

After standing sixteen hours at room temperature (20°) 1 cc. $\frac{N}{10}$ sodium hydroxide was added to each tube with the following results:

(5) Colorless.

(6) Yellowish.

(1), (2) and (3) were of about the same tint, namely, very faintly pink.

(4) Much deeper pink, and when compared with (1), (2) and (3) in the Duboscq-Soleil colorimeter it was found to be just three times as pink as (1), (2) and (3).

A few experiments performed with sodium iodoxybenzoate did not indicate that the oxygen of this substance is capable of furnishing oxygen in this reaction. The results are not conclusive, however, and it is our intention to return to this problem as soon as the opportunity presents itself. The problem is one of considerable interest in itself, but we do not wish to attach too much importance to it so far as the physiological availability of the oxy-

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gen is concerned. It is perfectly conceivable that a substance which cannot furnish oxygen for this reaction may still be utilized as an oxidizing agent by cells for physiological oxidations and the reverse of this is also probably true. The study justifies itself because it is the only evidence which we can obtain in test-tube experiments of the probably physiological availability of the oxygen in any given compound.

The effect of sodium iodosobenzoate and iodoxybenzoate on the blood: In this part of the work we have been assisted by Dr. C. H. Bunting, who made all of the blood counts. He counted the leucocytes, the erythrocytes, and, in some cases, also the platelets. We desire to express our thanks to him for his valuable help. We have made the haemoglobin determinations ourselves using the Miescher apparatus.

The drugs were injected by placing a canula into the external jugular vein of rabbits under local anaesthesia and as nearly aseptically as possible. The insertion of the canula was rendered necessary because it was desired to give the drugs slowly and in considerable volume, conditions which preclude ear vein injection.

Experiment 2. Rabbit, male, 1790 grams, 15 c.c. of $\frac{N}{30}$ sodium iodosobenzoate were injected during a period of twenty-nine minutes

	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	COAGULATION TIME
	per cent			
Five hours before injection..	93	6,384,000	9,000	3 minutes
Injected 15 cc. $\frac{N}{30}$ sodium iodosobenzoate.....				
Twenty hours after injection	73	6,016,000	19,000	4 minutes
Forty-three hours after injection.....		5,600,000	11,000	3 minutes 30 seconds
Sixty-eight hours after injection.....	72	5,360,000	11,000	3 minutes 15 seconds

Professor Bunting also made differential counts, the results of which were as follows:

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	NOVEMBER 3 (BEFORE INJECTION)		NOVEMBER 4		NOVEMBER 5		NOVEMBER 6	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Polymorphonuclears	2160	24.0	11362	59.8	4422	40.2	4246	38.6
Eosinophiles	180	2.0	76	0.4	176	1.6	154	1.4
Basophiles	288	3.2	380	2.0	330	3.0	176	1.6
Small mononuclears	5922	65.8	6422	33.8	4818	43.5	5390	49.0
Large mononuclears	450	5.0	760	4.0	1254	11.4	1034	9.4
Totals	9000		19000		11000		11000	

The large leucocytosis noted in this rabbit is thus shown to be due almost entirely to the increase in the polymorphonuclear leucocytes which after twenty-four hours were found to be increased more than five times. On November 5, forty-four nucleated red cells were found indicating blood regeneration.

With this large amount of sodium iodosobenzoate, corresponding to 0.132 gram of free iodosobenzoic acid, there was some blood destruction although this was not severe.

At the completion of the experiment the rabbit was killed by a blow on the neck and an autopsy was done. The only noteworthy finding at autopsy was the congestion of both lungs at the base. In the right lung there was a triangular haemorrhagic area of considerable size. The condition of the lungs was probably sufficient to account very largely for the degree of anaemia noted.

Experiment 3. Rabbit, albino, female, 1500 grams. In a period of 32 minutes 15 cc. $\frac{N}{30}$ sodium iodosobenzoate were injected into the external jugular vein

	HAEMOGLOBIN	LEUCOCYTES	ERYTHROCYTES	COAGULATION TIME
	per cent			
Before injection	86	10,000	6,224,000	2 to 2½ minutes
Fifteen cc. $\frac{N}{30}$ sodium iodosobenzoate injected				
Twenty-two hours after injection		13,500	6,032,000	2 minutes
Seventy hours after injection		12,500	6,480,000	1½ to 2 minutes
One hundred and twenty-five hours after injection	85			

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In this case practically no blood destruction was noted, although the same dose was used as in Experiment 2 and the rabbit was smaller. The leucocytosis in this case was much less marked than in the previous one.

In Experiment 1 in which a male rabbit of 1800 grams was used the platelet count fell from 670,000 to 470,000 within $3\frac{1}{2}$ hours after injecting 30 cc. of a $\frac{N}{80}$ solution of the iodosobenzoate. The ratio of platelets to red cells fell from 1:7.5 to 1:10 during the interval. There was no oedema of the lungs.

Experiment 5. Rabbit, female, 2100 grams

	ERYTHROCYTES	LEUCOCYTES
Before injection	5,400,000	17,000
Injected 10 cc. $\frac{N}{33\frac{1}{3}}$ sodium iodoxybenzoate		
Nineteen hours after injection	5,000,000	25,000
Forty-two hours after injection	5,416,000	17,500

No nucleated reds appeared in the circulating blood. The rabbit remained entirely well. Many other experiments gave similar results. The conclusions to be drawn regarding the effect of the substances on the blood when injected intravenously are as follow:

1. Both sodium iodosobenzoate and iodoxybenzoate usually cause a more or less marked leucocytosis affecting especially the polymorphonuclear leucocytes.

2. Iodosobenzoate, when injected in quantities of 15 cc. $\frac{N}{80}$ solution in rabbits causes a certain decrease in the red cells in the haemoglobin. In many cases the destruction is entirely insignificant, and in no case was it great.

3. After iodosobenzoate in large doses rabbits often show a haemorrhagic condition of the lungs. There may be simply a very marked congestion or in some cases haemorrhage into the lung tissue may be noted.

As further evidence of the extreme degree of congestion of the lungs, which may occur in some cases following iodosobenzoate, we have frequently noted in cats, that just before death there escapes from the trachea very large amounts of a blood stained

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frothy fluid. In other cases neither oedema nor congestion of the lungs was found at autopsy.

THE EFFECT OF SODIUM IODBENZOATE, IODOSOBENZOATE, AND IODOXYBENZOATE ON THE CIRCULATION AND RESPIRATION

In order to elicit the effect of these substances on the circulation and respiration it is necessary that they be given intravenously. The reason for this is easily seen from what follows. The activity of both iodoso- and iodoxybenzoate is due to the presence of active oxygen—the reduction product is quite inactive in corresponding doses. In their passage into the blood stream either from the gastro-intestinal tract or from the subcutaneous tissues, much or all of this active oxygen is lost. Hence in discussing the action of the substances on the circulation and respiration we refer in all cases to experiments in which the salts are given intravenously.

Action on the circulation

As previously pointed out, sodium iodbenzoate does not contain active oxygen and it therefore furnishes a valuable control in studies of iodoso- and iodoxybenzoate. It will be remembered that iodosobenzoic acid is an exceedingly weak acid, whereas iodoxybenzoate acid is quite strong. Furthermore these two substances behave quite differently in certain chemical reactions. Thus Hartmann and Meyer found that iodosobenzoic acid on treatment with alkalis yields salicylate, whereas iodoxybenzoic acid, although richer in oxygen, yields the salts of benzoic and iodic acids, but no salicylate. Hence iodoso- and iodoxybenzoates differ widely from one another in their general chemical properties. They have one chain of properties in common, namely, those dependent on the presence of chemically very active oxygen. On the other hand, iodbenzoic acid differs from the other substances principally in that it contains no active oxygen. Hence any pharmacological property possessed by both iodoso- and iodoxybenzoates and not possessed by iodbenzoate may fairly be attributed to the active oxygen which the former substances contain.

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Sodium iodbenzoate: With the vagi intact the injection of 5 cc. of $\frac{N}{10}$ sodium iodbenzoate in the cat or rabbit usually causes a rise of arterial blood pressure, which, though not large, lasts for some minutes (see fig. 7). The rise is usually 8 to 10 mm. of mercury, although a rise of as much as 20 mm. was noted in some cases. Sometimes there is no increase in the blood pressure but the point of interest is that it never causes a fall of blood pressure —at least it has never occurred in our experiments. At no time during its action does it cause any change in the pulse rate. The very slight decrease in the pulse rate in one experiment was probably secondary to the considerable rise of blood pressure. With the blood pressure high as the result of vagal section, iodbenzoate does not cause a rise of blood pressure. The harmless character of the salt when injected intravenously stands out in marked contrast to the other salts. In an experiment on a cat 25 cc. of the $\frac{N}{10}$ solution were injected before cutting the vagi, and the same amount after cutting the vagi, corresponding in all to 0.64 gram of free iodbenzoic acid and at the end of the experiment the blood pressure was 180 mm. and the pulse rate 238. At the beginning of the experiment, with the vagi intact, the blood pressure was 149 mm. and the pulse rate 208. This indicates how bland the salt is when injected intravenously. We have not determined the factors in the rise of blood pressure, but certain facts would indicate that it is probably largely due to increased cardiac output.

Sodium iodosobenzoate: We studied the action of this substance in a large number of experiments using cats, rabbits and dogs. The action of the salt on the circulation is essentially the same in all three animals. We shall describe the typical action of the salt first and then deal with the exceptions. The dose required to elicit the typical action varies somewhat in different animals, even of the same species.

In the cat the first indication of the typical effect of the salt on the circulation is seen when 1 cc. of the $\frac{N}{10}$ solution (corresponding to 0.0132 gram of the free acid,) is injected intravenously. It may be necessary to repeat this dose in order to elicit the action

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of the salt to even a slight extent. The full activity of the salt is seen after the injection of 5 cc. $\frac{N}{20}$ solution (0.066 gram of the free acid). In this case there is at first a sudden fall of blood pressure of often 40 mm. of mercury within 10 to 20 seconds following the injection. The blood pressure then continues to fall, but more gradually for the next minute or more, during which period it may show a further fall of 10 to 20 mm. of mercury. The blood pressure recovers very slowly. There is no change in the pulse rate at any time. In order to determine the factors in this fall of blood pressure we have studied the effect of the salt: (1) On the volume changes of the heart using a cardio-plethysmograph connected with a piston recorder. (2) On the volume of a loop of the intestines before and after cutting the splanchnics. (3) On the rate of outflow from the mesenteric vein on perfusing the isolated intestines using the Wiggers outflow recorder.

1. *Studies with the cardioplethysmograph.* The injection of 3 cc. $\frac{N}{20}$ sodium iodosobenzoate into the saphenous vein (cat) caused a marked fall of blood pressure. During the time that the principal fall of blood pressure occurred there was practically no change in the rate or amplitude of the heart beat. After about one minute following the injection, and while the blood pressure was still falling, there was noted a loss of tone affecting systole much more than diastole and resulting, at the height of the effect, in a decrease of about 15 per cent in the amplitude of the beat. The heart rate dropped from 36 to 34 in ten seconds. This effect was transitory. Following this the amplitude gradually increased until it was about 15 per cent above the normal and the rate fall to 31 in ten seconds. It required from $2\frac{1}{2}$ to 3 minutes for the heart to regain its previous tone. A second injection of 3 cc. $\frac{N}{20}$ solution again caused a marked fall of blood pressure. Here also, after the principal fall of blood pressure had occurred, the heart became dilated, the amplitude decreased markedly and the beat became irregular. The dilatation became extreme four minutes after the injection and remained so for about two minutes, when it slowly recovered and the heart beat became almost normal again. During the period of cardiac dilatation the blood pressure fell to 16 mm. Figs. 1 and 2 reproduce tracings from

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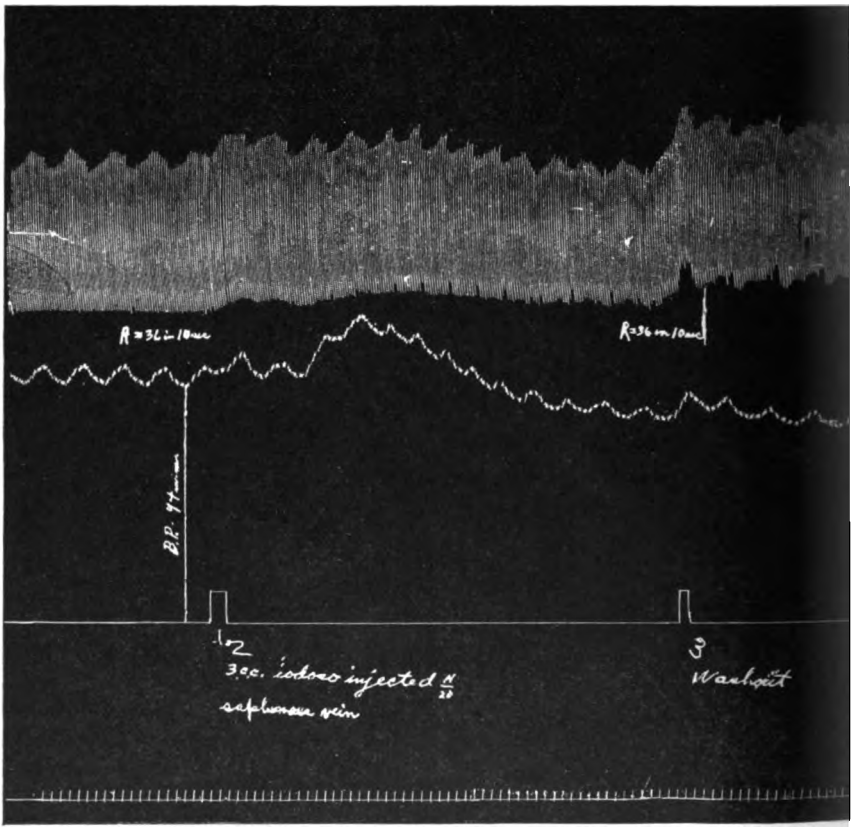
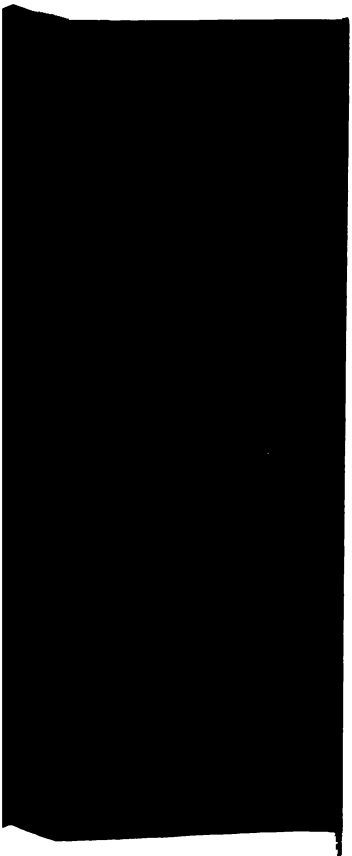


Fig. 1. *a*, (upper tracing) Tracing from cardioplethysmograph, piston record into saphenous vein. 3, Physiological salt solution injected to clear canula. Rea

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6, Blood pressure from c
left to right. (Reduced.)

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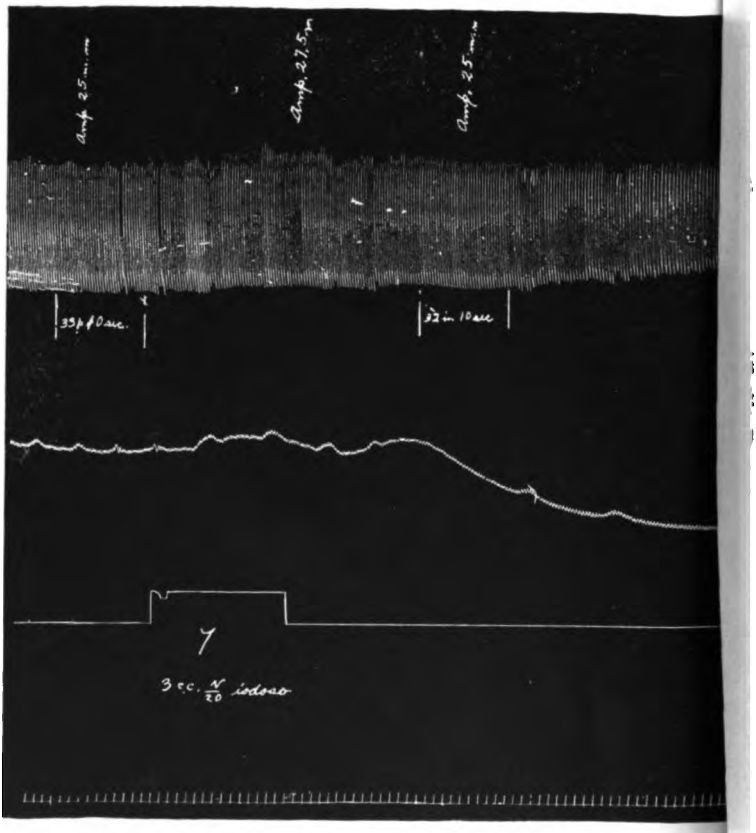


Fig. 2. From same experiment as figure 1. At 7, 3 cc. $\frac{N}{20}$ sodium

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which the above description was take
by other experiments.

It is impossible to say whether or
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continued fall of blood pressure
rather precipitous fall. It may be
is the result, rather than the cause,
pressure and the increase in the ve
the dilatation of the peripheral vesse
cardiac condition and the rise of bl
neously. The fact that most of the
before the efficiency of the heart is
indicate that the decreased efficiency
are the result of the fall in the arteri
pressure. The conclusion from the
pressure under sodium iodosobenzo
cardiac in origin.

2. The effect of the salt on the
intestine before and after cutting the
that the fall of blood pressure is du
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caused the volume of the loop of in
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the volume of the loop ran parallel
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3. In order to preclude further a
wall, the intestines were perfused th
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case did the salt have the slightest
The initial fall of blood pressure is
of the vaso-motor center.

Sometimes iodosobenzoate causes
The most marked case was in an exp

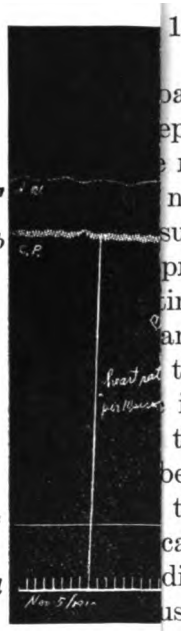


Fig. 3. Cat' an
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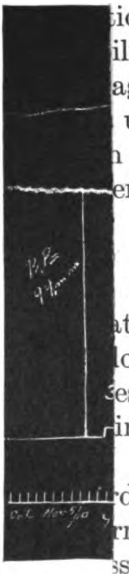
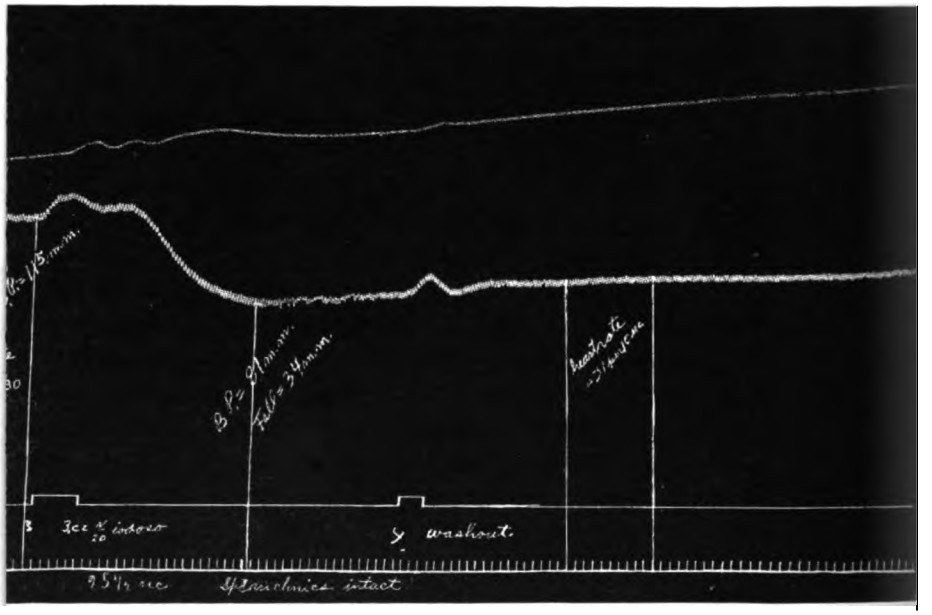
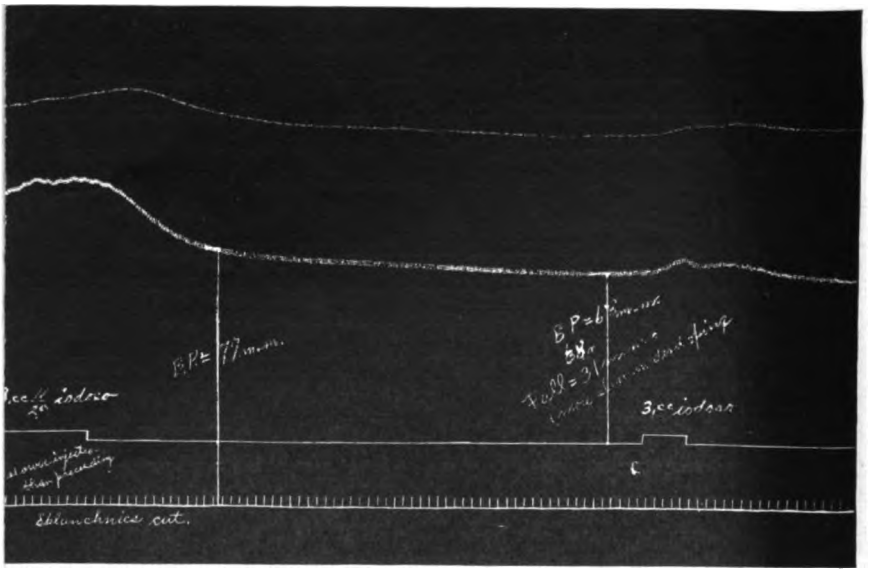


Fig. 4.
iodosoben

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, *a*, Volume of loop of intestine, piston recorder. *b*, Carotid pressure. *c*, Signal ressure. *d*, Time in seconds. At 3, 3cc. $\frac{N}{10}$ iodosobenzoate injected into femoral vein. tact. Read left to right. (Reduced to $\frac{2}{3}$.)



(From same animal as Fig. 3.) The splanchnics had been cut. At 7, 3. cc. $\frac{N}{10}$ zoate were injected into femoral vein.

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used. In this case the first injection of 5 cc. $\frac{N}{16}$ iodosobenzoate caused tremendous stimulation although the animal was deeply anaesthetized with ether. The struggling interfered with the recording apparatus so that the height of blood pressure was not recorded. However, when the struggling ceased the blood pressure on the return toward normal was 182 mm. compared with a previous normal pressure of 162 mm. The pulse rate at this time was 256, the normal being 194. A second injection of the same dose in this animal caused a rise of blood pressure from the normal of 156 mm. to 220 mm. of mercury, the pulse rate increasing from 176 to 216. Subsequent injections produced the typical fall of blood pressure. In a few cases the iodosobenzoate caused first a fall and later a considerable rise in the blood pressure. Our cardioplethysmographic records indicate that the secondary rise is probably due to increased cardiac output. When very small quantities of iodosobenzoate are used (1 cc. $\frac{N}{100}$ corresponding to 0.00264 gram of the free acid), an insignificant rise of blood pressure may be noted. The cause of the rise of blood pressure which is sometimes seen with the iodosobenzoate has not been determined. In the cases associated with struggling it is probably due to the muscular contraction. In other cases where there is no struggling it is in all probability cardiac in origin. We have always found that at certain stages of the action the cardiac output is increased, and when this is unusually large and not sufficiently antagonized by depression of the vaso-motor center, the net result may be a rise of the arterial blood pressure.

Sodium iodoxybenzoate: The action of this substance on the circulation is identical in its general characteristics with that of the iodosobenzoate. It is simply less powerful than the iodosobenzoate. The fall in the blood pressure in the typical cases is usually less marked and it causes a rise of blood pressure in a larger percentage of the cases than does the iodosobenzoate. It is not so depressing on the heart and an increase in the cardiac output is the rule. Again it requires more of this salt to permanently injure the heart. For these reasons the fall of blood pressure

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under iodoxybenzoate, even though it caused as large an initial fall is more rapidly recovered from than in the case of the iodosobenzoate. A typical experiment may be given. Five cubic centimeters of a $\frac{N}{10}$ solution were injected into the saphenous vein of a cat. The blood pressure fell within eighteen seconds from 120 to 70 mm. The blood pressure did not continue to fall from this point as in the case of iodosobenzoate but it required about ten minutes to return to normal. Cardioplethysmographic tracings showed that the fall of blood pressure is not due to any cardiac effect of the drug. Perfusion of the isolated intestines recording the rate of outflow proved that the salt does not act directly on the vessel wall and here again we conclude that the salt lowers the blood pressure by depressing the vaso-motor center We believe that the rise of blood pressure not infrequently seen, is due mainly to increased cardiac output, although we have not excluded the possibility that under certain conditions the salt may stimulate the vaso-motor center.

The respiration

By far the most interesting and significant action of these substances is on the respiration. As in the case of the circulation the salts must be injected intravenously in order to elicit their characteristic effect.

The respirations were recorded by several methods and frequently two methods were used in the same experiment. In most cases the respiration was recorded by a tambour connected with the trachea tube, and in addition in the experiments with rabbits, Head's method of recording the respiration from the slip of the diaphragm was employed. In many of the experiments with cats the respiration was also recorded directly from the diaphragm by means of a thread connecting the diaphragm with a receiving tambour, which was in turn connected with a recording tambour, using air transmission.

Sodium iodbenzoate: In ordinary doses of 5 cc. $\frac{N}{10}$ solution or less this substance does not affect the rate or amplitude of the respiration in the cat, dog or rabbit. Hence in regard to its action on both the respiration and the circulation this substance is bland.

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Sodium iodosobenzoate and sodium iodoxybenzoate: These substances have practically identical effects on the respiration, so that they may be considered together. Their characteristic effect on the respiration is the production of apnoea.[13] The respiration always stops in repose, passive expiration. Apnoea may be produced by the salts either with the vagi intact or cut. The latter condition, however, favors the production of apnoea. Extremely long periods of apnoea were obtained with the vagi cut. The interval between the injection and the production of apnoea, as well as the duration of the apnoea, depends upon the dose and apparently upon other factors of which we are entirely ignorant. In some of our experiments, apnoea has occurred immediately at the completion of the injection of the salt—the injection having lasted 10 to 12 seconds. In one case the apnoea began seven seconds after an injection requiring two seconds. In other cases the interval was much longer, varying between eight seconds and four minutes in our series. One of the most interesting cases of a long interval between the injection and the production of apnoea was the following, which also illustrates the fact that it is easier to produce apnoea with the salts when the vagi are cut than when they are intact.

Experiment 14. A cat (3400 grams) received four injections of $\frac{N}{20}$ sodium iodoxybenzoate of 2.5, 2.5, 5 and 5 cc. respectively within a period of 8½ minutes without more than a slight depression of the respiration. Seventeen minutes later the vagi were cut and about three minutes later there occurred an apnoea which lasted ninety-seven seconds. This apnoea began twenty minutes after the last injection.

The duration of apnoea in our series of twenty-five experiments

[13] The propriety of using the term "apnoea" in this connection will probably be questioned. The term really means a temporary cessation of the respiration. Miescher distinguished two forms of apnoea designating that form due to changes in the gaseous composition of the blood as "apnoea vera" and that due to the stimulation of the vagus and the sensory nerves of the larynx and nasal mucosa as, "apnoea spuria." The same chemical changes in the cells of the center may condition both forms of apnoea. We here use the term apnoea because we believe the cause of the respiratory stoppage to be essentially the same as in apnoea vera. The reasons for this conclusion will be discussed in a later paper by one of us.

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varied from a few seconds up to one hundred and fifty-one seconds. Many of the records show an apnoea of from 1 to 2 minutes. In some experiments apnoea did not occur. By starting with small doses it can be obtained in the cat and rabbit almost invariably. Apnoea can also be produced in the dog, but large amounts of the substance may be required, and therefore we have not used dogs in many experiments. To illustrate how much of the salts dogs will stand, we may cite Experiment 5, in which a dog of 6.5 kilos received 247 cc. of a $\frac{N}{26.4}$ solution of sodium iodosobenzoate within a period of $1\frac{1}{2}$ hours. This caused a respiratory pause of only four seconds. The respiratory rate was decreased. This quantity of the salt did not kill the animal. In Experiment 4 a dog of 10.1 kilos received 153 cc. of a $\frac{N}{26.4}$ and 25 cc. of a $\frac{N}{13.2}$ solution of sodium iodosobenzoate. There occurred a respiratory pause of thirty-seven seconds. Death in this case was due to the salt. The heart continued to beat forty-three seconds after the respiration stopped.

Death under these substances is due almost invariably to failure of the respiration, the heart continuing to beat from thirty seconds to five minutes after the respiration ceases.

The production of apnoea by the salts is entirely independent of blood pressure changes. They were obtained with a rise of blood pressure in some cases and with a fall of blood pressure in others. Sometimes the injection of the salts is not followed by apnoea, but the depression of the respiration is merely indicated by a decrease in the rate and depth of the respiration.

So far we have only spoken of respiratory depression, but we also have abundant evidence of stimulation of the respiration by the salts. The primary effect is occasionally an increase in the rate and depth of the respiration. This usually occurs after large doses and threshhold doses seldom cause dyspnoea.[14]

Frequently the injection of a good sized dose, such as 5 cc. $\frac{N}{20}$ solution, causes for a very brief period a decrease in the rate and depth of the respiration immediately following the injection and lasting but a few seconds to be replaced by extreme dyspnoea.

[14] There is great variation in the dose required to produce the same effect in different animals.

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A probable explanation of this apparently paradoxical result will be considered in a later paper by one of us, dealing with a theory of the respiration. A few typical examples of the effect of the salts on the respiration may be given.

In cats and rabbits the threshhold dose usually required to produce an effect on the respiration, may be placed at 1 or 2 cc. $\frac{N}{100}$ solution, which corresponds to 0.00264 to 0.00528 gram of

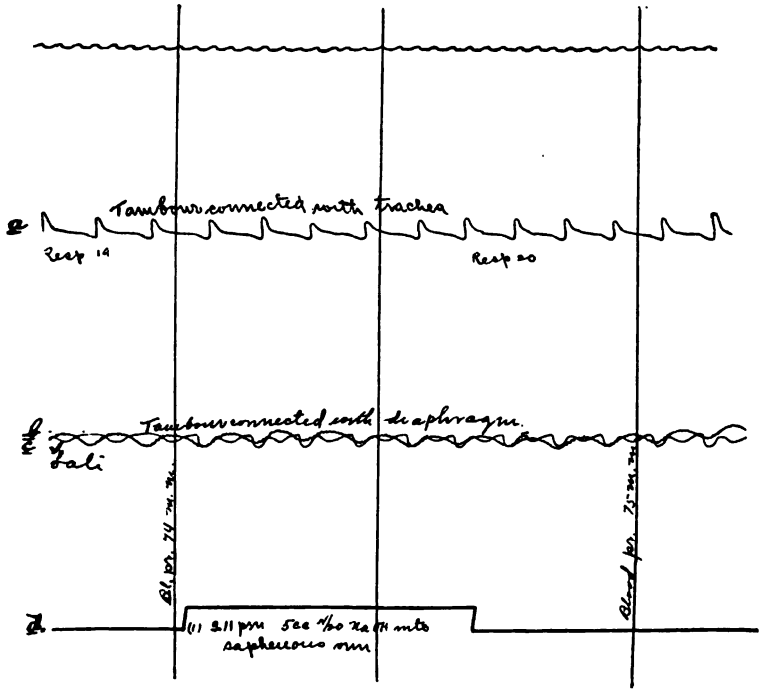


Fig. 5. At 3.11, 5 cc. $\frac{N}{20}$ NaOH injected into saphenous vein. Tracings same as in Fig. 6.

the free acid. In some animals 1 cc. or more of $\frac{N}{20}$ solution may be required to elicit the effect. In a cat of 4.3 kilos (Experiment 24) the injection of 1 cc. $\frac{N}{100}$ solution of iodosobenzoate was followed ten seconds later by a respiratory pause of five seconds, and thirty-five seconds later another pause of five seconds occurred. The respiratory rate was decreased from 32 to 28 per minute and remained below normal until the next injection was made.

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The amplitude of the respiration was very slightly increased. Dyspnoea did not follow this injection. The effect was purely a depression of the respiration. Two minutes after this injection a second injection of 2 cc. $\frac{N}{100}$ iodosobenzoate was made. Seven seconds later there occurred an apnoea of 14½ seconds duration. This dose was followed by a rise of blood pressure (114 to 120 mm.) most of which occurred during the apnoea. Following the apnoea there was a very slight increase in the rate and depth of the respiration. There was no dyspnoea.

A cat of 2400 grams (Experiment 13), in which the vagi had been cut received at 4:13 p.m. 5 cc. $\frac{N}{20}$ iodosobenzoate. There occurred several long pauses in the respiration immediately following the injection. After it became more regular the rate was reduced from 20, the normal, to 16 per minute. At 4:16, 3 cc. $\frac{N}{20}$ iodosobenzoate were injected. This further reduced the respiratory rate to 13. At 4:17½, 5 cc. $\frac{N}{20}$ iodosobenzoate were injected. The respiratory rate, which had returned to 20 was immediately reduced to 14, and gradually diminished in size until after seventy-four seconds an apnoeic period of twenty seconds occurred. Respiration was then resumed, at first of fair depth but gradually diminishing in rate and size. After 11 respirations within fifty-six seconds a period of apnoea lasting twenty-five seconds occurred, followed by a single shallow respiration and then apnoea of eighty-six seconds. The blood pressure remained above 50 mm. until the last few seconds when it dropped a few millimeters. Following the long apnoea, there gradually developed most extreme dyspnoea, lasting about two minutes when the respiration finally ceased, the heart continuing to beat one hundred and sixty-four seconds after the last respiration. This experiment illustrates fatal apnoea under the salt, and closely resembles the fatal apnoea described by Henderson[15] but produced by apparently entirely different means. We shall try to show later that the mechanism is probably essentially the same. It is very difficult to determine whether iodosobenzoate or iodoxybenzoate acts the more powerfully on the respiration, as it is difficult to get comparable experiments in the same animal since the animal seldom responds to the same extent

[15] Amer. Jour. of Physiol, 25, 310 (1910).

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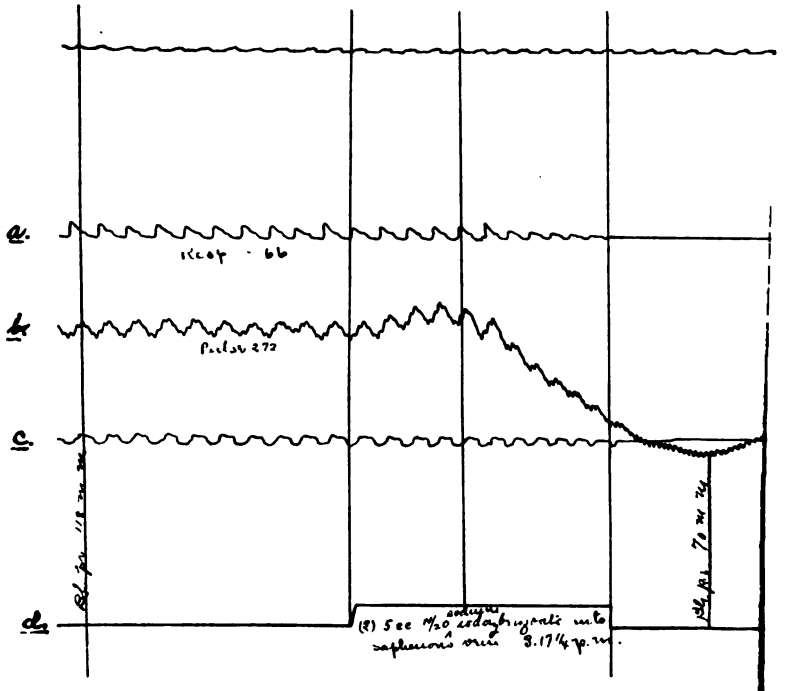
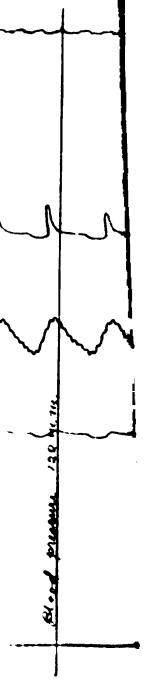


Fig. 6. Cat, male, 2100 g. ether, vagi intact. Upp
d, Signal pen and zero pressure.
At 3.17¼ P.M. (point indicated) 5 cc. $\frac{N}{10}$ sodium iodoxybe



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to successive doses. In some animals an increased susceptibility and in other animals a decreased susceptibility to the second dose is noted. The effect of iodosobenzoate in depressing the circulation so much more than iodoxybenzoate, tends to enhance its effect upon the respiration.

The great difference noted between the effects on the respiratory and vaso-motor centers of iodbenzoate on the one hand, and

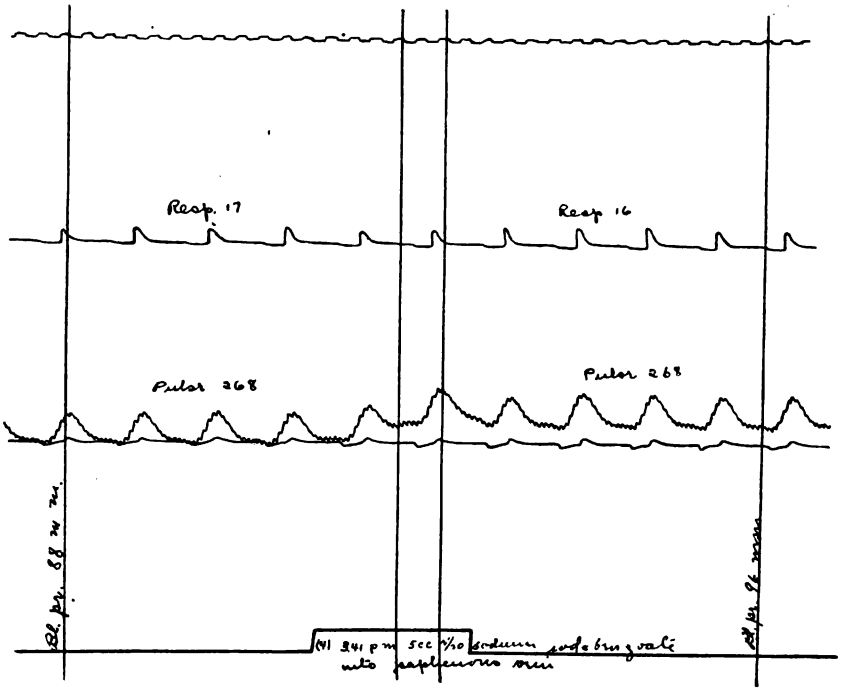


Fig. 8. From same experiment as Figs. 5, 6 and 7. At 3.41 P.M. 5.cc. $\frac{N}{20}$ sodium iodbenzoate injected into saphenous vein.

iodosobenzoate and iodoxybenzoate on the other, must be attributed to the active oxygen which the latter substances contain. As previously pointed out, the latter substances differ from iodbenzoic acid in the possession of this active oxygen. Iodosobenzoate and iodoxybenzoate have the same effect on the respiratory center and yet they differ quite markedly from one another chemically. The common pharmacological activity must be due to

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their common chemical potentiality, namely, the presence of active oxygen in available form. In the following experiment (No. 17) it would appear that the iodoxybenzoate is the more powerful in its effect upon the respiration. In this experiment 5 cc. $\frac{N}{10}$ sodium hydroxide was first given as a control to exclude any possible alkali effect, then 5 cc. $\frac{N}{10}$ sodium iodoxybenzoate were given at 3:17¼ p.m. At 3:33 p.m. 5 cc. $\frac{N}{10}$ sodium iodosobenzoate were injected and at 3:41 5 cc. $\frac{N}{10}$ sodium iodbenzoate. The animal used was a female cat weighing 2100 grams. The vagi were intact. The record speaks for itself and requires no comment. (Fig. 5, 6, 7 and 8.) We shall consider certain other substances which cause temporary cessation of the respiration in the communication following this one.

SUMMARY

1. Improved methods for making and purifying iodbenzoic, iodosobenzoic, and iodoxybenzoic acids are described.

2. The local action of the free acids and their sodium salts are described. The results show that of the sodium salts, those of iodbenzoic and iodoxybenzoic acids are mild, while that of iodosobenzoic acid is extremely irritating when injected subcutaneously or intraperitoneally.

3. It is shown that sodium iodosobenzoate and iodoxybenzoate, in dilute solution, have a peculiar metallic taste very much like that of hydrogen peroxide and it is suggested that the active oxygen confers upon all of these compounds their peculiar taste.

4. Solutions of sodium iodosobenzoate and iodoxybenzoate immediately oxidize haemoglobin to oxyhaemoglobin.

5. The peroxidase of the blood enables sodium iodosobenzoate to oxidize phenolphthalin to phenolphthalein; in other words, it can furnish oxygen for this peroxidase reaction in the same manner that hydrogen peroxide does.

6. Sodium iodosobenzoate and sodium iodoxybenzoate, when injected intravenously, do not exercise a destructive action on the blood to any extent. Leucocytosis of a moderate grade may be noted.

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7. In order to elicit their characteristic effects on the circulation and respiration the salts must be injected intravenously.

8. On the Circulation: *Sodium iodbenzoate* is but very slightly active, causing a rise of arterial blood pressure. The mechanism by which this is brought about was not determined.

Sodium iodosobenzoate usually causes a marked and long continued fall of blood pressure. During the time that the blood pressure shows the greater part of the fall, the heart rate and amplitude are hardly affected. The salt causes dilatation of a loop of intestine when the splanchnic nerves are intact, but not after they are cut. It does not affect the caliber of blood vessels by peripheral action. Hence it depresses the vaso-motor center. At certain stages the salt increases the cardiac output and we believe this to be the mechanism involved in the rise of blood pressure occasionally noted.

Sodium iodoxybenzoate acts similarly to the iodosobenzoate on the circulation, but its action is not so marked. It causes a rise of blood pressure in a larger per cent of the cases than iodosobenzoate and is less depressing on the heart. The usual fall of blood pressure is due to depression of the vaso-motor center.

9. On the Respiration: Sodium iodbenzoate contains no active oxygen and in the doses we employed it does not affect the rate or depth of the respiration. Sodium iodosobenzoate and sodium iodoxybenzoate have about the same effect on the respiration. They both cause cessation of the respiration in repose, in dogs, cats and rabbits. The best results are obtained with cats. The period of apnoea usually begins within a few seconds after starting the injection and lasts from a few seconds up to 2 or 3 minutes and is terminated by spontaneous return of the respiration. Apnoea may come on several minutes after the last injection. Apnoea is more easily produced by the drugs after section of the vagi, but this is not essential. Apnoea may occur with a rising or a falling blood pressure and is independent of it. During the apnoea there is usually a fall of blood pressure, while the pulse rate remains normal.

10. Of the medullary centers, the respiratory center is the most sensitive to sodium iodosobenzoate and iodoxybenzoate.

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The vaso-motor center is next and the cardio-inhibitory center is not affected by them.

11. It is pointed out that the remarkable effect of sodium iodosobenzoate and iodoxybenzoate on the respiration and also their effect on the vaso-motor center, must be due to activity of the oxygen combined with the iodine in these compounds.

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THE ACTION OF HYDROCYANIC ACID ON THE RESPIRATION AND THE ANTAGONISTIC ACTION OF SODIUM IODOSOBENZOATE[1]

W. E. GROVE AND A. S. LOEVENHART
From the Pharmacological Laboratory of the University of Wisconsin

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Hydrocyanic acid is known to depress physiological oxidations as was first proved by the classical work of Geppert.[2] Many investigators, beginning with Schönbein,[3] have shown that hydrocyanic acid inhibits the activity of the oxidizing enzymes. This is the only fundamental chemical process occurring in living matter which is known to be influenced by this substance and since its physiological and toxicological effects can be readily and satisfactorily explained on the basis that its effects are due to depression of physiological oxidation, it would be a purely gratuitous assumption to suppose that its effects result from changes in any other chemical activity in the cells. We therefore conclude, that in the light of present knowledge, the effects of hydrocyanic action on the function of any cell is the expression of the reaction of that cell to diminished oxidation. Therefore if sodium iodosobenzoate is capable of accelerating physiological oxidation we should expect to find an antagonism between hydrocyanic acid and iodosobenzoate.

The effect of hydrocyanic acid on the respiration is now universally recognized as central. It is unaffected by section of the vagi or by the administration of atropine.[4] It is well known that hydrocyanic acid is one of the strongest stimulants for the

[1] The expenses of this research have been partly defrayed by a grant from the Rockefeller Institute for Medical Research.

[2] Zeitschrift f. klin. Med., 15–208, 307 (1889).

[3] Zeitschrift f. Biol., 3–140, 325 (1867).

[4] Boehme and Knie: Arch. f. exper. Pathol. und Pharmacol., 2, 129 (1874).

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respiratory center.[5] The injection of threshhold doses intravenously increases the rate and depth of the respirations. When larger quantities are injected there is often noted a slowing of the respiratory rate and a great increase in the depth of the respirations. Still larger quantities cause depression and paralysis of the respiration and death soon ensues. These results have been obtained by a great many investigators and are among the most constant responses of the vital mechanism to drugs. We have, however, noted an interesting phenomenon in the response of the respiratory center to hydrocyanic acid to which attention has not previously been called.

When somewhat more than the threshhold dose of hydrocyanic acid is injected intravenously into rabbits we have noted that there is complete stoppage of the respiration for periods varying in our experiments from a few seconds up to 230 seconds and terminating with spontaneous return of the respiration. The rabbits were anaesthetized by giving 0.1 gram chloral and 0.5 gram urethane per kilo subcutaneously and ether was used during the operative procedures. The respiration was recorded by means of a tambour connected with the tracheal tube by means of a T-tube and in most of the experiments Head's method of recording the respiration from the isolated strip of the diaphragm was also employed.

In order to obtain complete temporary stoppage of the respiration, it is necessary to inject from 0.5 to 1 cc. $\frac{N}{15}$ solution per kilo of hydrocyanic acid or sodium cyanid (about 1 mg. hydrocyanic acid or about 2.0 mg. sodium cyanid). The injection should be made rapidly, and it is better to plunge the needle of the hypodermic syringe directly into the vein rather than to connect the syringe to the vein by means of a canula, as the latter delays the injection and requires washing out. In most cases the stoppage occurs in passive expiration but it may occur in any phase of the respiration. If it does occur in active inspiration or expiration the position of passive expiration is gradually assumed during the stoppage. It is difficult or impossible to

[5] Heinz: Handbuch der experimentellen Pathologie und Pharmakologie. Jena, 1906, vol. 2, p. 588.

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bring on the respiratory pause without at least a very brief preliminary stage of stimulation. If a small portion reaches the respiratory center first it results in a stage of stimulation, the duration of which depends on the time required for the full dose to reach the center. In order to produce the temporary paralysis it is necessary for the full dose to be injected within a few seconds. The larger the dose, up to the lethal dose, and the more rapidly it is given, the longer will be the respiratory pause. Respiratory paralysis of 1 to 2 minutes duration can be obtained almost without exception. The temporary paralysis of the respiration is independent of the vagi as it occurs whether they are intact or cut. The type of respiration noted on recovery from the paralysis varies somewhat with different individuals. In some cases the first respirations are very small and gradually increase in size until they are many times larger than the normal respirations. During this period the rate is very slow. The respirations then gradually decrease in size and increase in rate until the normal is reached. The return to normal requires 15 to 20 minutes or more. The circulatory phenomena associated with the period of apnoea are interesting and important in the interpretation of the mode of action of hydrocyanic acid. Within 2 to 5 seconds following the injection the heart rate is very much decreased, due in part to stimulation of the cardio-inhibitory apparatus centrally, and in part to direct action on the heart. This is shown by the fact, that if both vagi are cut during the stage of exceedingly slow heart rate, the heart rate increases markedly but does not return to the normal rate. Hence the cardio-inhibitory center is largely but not entirely responsible for the slow heart rate. If atropine is now given in quantities sufficient to paralyze the vagal endings the heart rate does not increase. Hence the residual slowing must be attributed to the direct action of hydrocyanic acid on the heart.

The slow pulse may continue throughout the period of respiratory paralysis and for three or four minutes after the return of the respiration when it gradually becomes normal.

In spite of the great slowing of the heart rate the blood pressure rose 40 mm. in one case due to stimulation of the vaso-motor

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center. In another experiment the blood pressure, after a very small and transitory rise, fell 40 mm. indicating that the vaso-motor center, like the respiratory center, was much depressed. The return to normal was very gradual and required several minutes. The work clearly shows that the respiratory center is much more sensitive to hydrocyanic acid than the cardio-inhibitory or the vaso-motor centers. In threshhold doses the respiration is affected before the other centers show any response whatever. Larger doses are usually required to slow the heart than to stimulate the vaso-motor center. When excessive doses are used the respiration, having passed through a short stage of stimulation, is paralysed temporarily while the vaso-motor center and cardio-inhibitory center are still in the stage of stimulation. The relative sensitiveness of these three centers to hydrocyanic acid is of much interest as it corresponds exactly with what has been found when the medulla is rendered anaemic.[6]

In connection with the power of hydrocyanic acid to cause temporary stoppage of the respiration it is of interest to review the action of other substances which have a similar effect.

A number of substances are known which cause temporary stoppage of the respiration. Tappeiner[7] in 1896 studied the action of phenylmethyl-isoxazol-chlormethylate and also that of certain pyrazol derivatives on the circulation and respiration. The first mentioned substance causes a temporary stoppage of the respiration, lasting in one case as long as 80 seconds. Tappeiner states that the respiration stops in active expiration.

Each injection is followed by a very marked slowing of the pulse rate which ceases after section of the vagi and is therefore due to stimulation of the vagal center. The blood pressure always rose from 10 to 20 mm. following each injection, due to stimulation of the vaso-motor center which more than compensated for the slowing of the pulse. Tappeiner found that the effects of the drug resemble the reflex described by Kratschmer.[8] Kratschmer showed that the injection of irritating vapor into

[6] J. A. E. Eyster: Jour. of Exp. Med., 8, 565 (1906).
[7] Arch. f. exp. Path. u. Pharmacol., 37-325 (1896).
[8] Sitzungsberichte d. Wiener Akad., 62-147 (1870).

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the nostrils of the rabbit causes cessation of the respiration in active expiration, a rise in the arterial blood pressure and a slowing of the pulse. Tappeiner used only rabbits in his studies and found that cocainization of the nasal mucosa prevents the drug from exhibiting its characteristic effects. He concluded that the action of phenylmethyl-isoxazol-chlormethylate is peripheral, causing a specific stimulation of the nerve endings of the ophthalmic branch of the fifth nerve in the nasal mucosa.

Jodlbauer,[9] in Tappeiner's laboratory, studied the action of tetramethyl-ammonium chloride given intravenously to rabbits and found that it causes a respiratory stoppage of 4 to 15 seconds duration, depending on the dose. He showed that the stoppage occurs in strong expiration and can be prevented by cocainizing the nasal mucosa. There is stimulation of the vagus centrally and peripherally and of the vaso-motor center.[10] The Kratschmer reflex fails entirely in dogs and is incomplete in cats and he finds that the drug cannot cause cessation of the respiration in either dogs or cats. He therefore concludes that tetramethyl-ammonium chloride acts by stimulating the endings of the ophthalmic branch of the trigeminus as had been previously found by Tappeiner for the quaternary base investigated by him. Pohl[11] found that certain quaternary papaverin bases injected intravenously cause stoppage of the respiration in expiration. He states that soon after the respiratory stoppage asphyxial phenomena occur, vagal pulse, fall of arterial blood pressure and convulsions, and the animal rapidly dies. If the dose is small the respiratory pause is only short and respirations return at first shallow but gradually become normal. He found that the stoppage of the respiration occurs after cocainizing the nasal mucosa and after sectioning the ophthalmic branches of the trigeminus on both sides. Hence he concludes that the drug acts centrally. He found that tetramethyl-ammonium chloride also causes stoppage of the respiration after section of the ophthalmic branch of the

[9] Arch. internation. de Pharmacodyn. et de Thérapie, 7–187 (1900).

[10] It is clear the stimulation of the vagal endings could not be due to the Kratschmer reflex.

[11] Arch. internat. de Pharmacodyn. et de Thérapie, 13–479 (1904).

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trigeminus on both sides and concludes that it acts centrally and not peripherally as found by Jodlbauer. Pohl's work leaves little room for doubt that these substances act centrally.[12] Marshall[13] found that protocatechyl-tropeine in doses of 0.005 to 0.01 gram causes paralysis of the respiration when injected intravenously into anaesthetized rabbits or cats. Complete stoppage usually occurred in 10 seconds after beginning the injection and lasted 4 to 20 seconds according to the dose. Frequently after 0.01 gram the respirations did not return. The respiration ceases in the expiratory phase. Marshall further found that protocatechyl-tropeine still causes the characteristic stoppage of the respiration after excision of the brain above the pons and excision of both fifth nerves. The drug is active in cats in which animals the Kratschmer reflex is not complete so far as the circulation is concerned, although they show stoppage of the respiration when irritating gases are blown into the nostrils. He concluded that this tertiary base acts centrally. In doses which cause respiratory stoppage, Marshall notes that all of these substances cause a decrease in the pulse rate, but he states that this is independent of the respiratory stoppage because they do not occur synchronously and the relative effects vary with different substances. A rise or a fall of blood pressure may be noted. On comparing the action of these drugs with that of hydrocyanic acid we see that they present the following points in common:

1. All cause temporary stoppage of the respiration in appropriate doses.
2. All are very toxic, death being due to respiratory failure.
3. All cause marked slowing and greatly increased amplitude of the pulse due partially to stimulation of the cardio-inhibitory center.
4. Doses required to cause temporary cessation of the respiration may cause a rise or a fall of blood pressure depending on the extent to which the stimulation of the vaso-motor center is capable of compensating for the decreased output of the heart.

[12] Quite recently Marshall has repeated the work of Pohl on tetramethyl ammonium chloride with the same results.

[13] Jour. of Physiol., 38; Proc. of the Physiol. Soc., lxxxiv, June 5, 1909.

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There is, therefore, a very close correspondence between the respiratory and circulatory effects of phenyl-methyl-isoxazol-chlormethylate, tetramethyl-ammonium chloride, the quaternary bases studied by Pohl, protocatechyl-tropeine and hydrocyanic acid. Hydrocyanic acid, in smaller doses than are required to produce temporary respiratory paralysis, is a powerful stimulant for the respiratory center. Jodlbauer notes that tetramethyl-ammonium chloride injected subcutaneously in rabbits increases the rate and depth of the respirations. He found that this substance acts on dogs and cats only as a respiratory stimulant. Very recently Marshall[14] has investigated the action of tetramethyl-ammonium chloride on the respiration. He found that it causes a transient stimulation of the respiratory center increasing both the rate and depth of the respirations in many decerebrated animals. He concludes that the temporary paralysis of the respiration under this substance is due mainly to a paralyzing action on the motor-endings of the respiratory muscles. Unfortunately it is not recorded whether the other drugs which temporarily paralyze the respiration, stimulate it when smaller doses are used. Tappeiner states that phenylmethylisoxazol-chlormethylate often causes convulsions during the respiratory paralysis. This is also occasionally seen when hydrocyanic acid is injected in the doses which we have recommended for producing temporary respiration paralysis. It would seem, therefore, to be highly probable that the other drugs mentioned act in the same manner as hydrocyanic acid, namely, by retarding physiological oxidation. We have found, however, that tetramethyl-ammonium chloride does not retard the direct guaiacum reaction of the extract of potato peel nor does it interfere with the guaiacum peroxidase reaction of human blood or saliva. It does not inhibit the catalytic decomposition of hydrogen peroxide by human blood. In all of these respects it differs markedly from hydrocyanic acid. If this substance does act by inhibiting oxidation in the cells of the medullary centers, either it must be specific for the oxidases of these cells, or it must act through an entirely different mechanism than the oxidizing enzymes.

[14] Journal of Physiol., 42; Proc. Physiol. Soc., p. xxxvii, June 3, 1911.

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THE ANTAGONISM BETWEEN HYDROCYANIC ACID AND IODOSOBENZOIC ACID

It is difficult to study antagonism to a substance like hydrocyanic acid which acts with such great rapidity, because the antagonist has to be administered either previously to or simultaneously with the hydrocyanic acid. Our method of studying the antagonism was as follows. Cats anaesthetized with ether were used in the study, as they proved to be better adapted to this work than dogs or rabbits. The blood pressure was recorded from the carotid artery. The respiration was recorded by a tambour connected with the tracheal tube and also directly from the diaphragm by taking a stitch in the diaphragm with a silk thread and attaching this to a receiving tambour. The movements of this tambour were registered by a recording tambour, air transmission being used. Canulas were inserted into the femoral veins on both sides. One canula was used for the injection of hydrocyanic acid and the other for the injection of sodium iodosobenzoate. Starting with very small doses and increasing gradually, the minimal amount of each substance required to produce its characteristic respiratory effects was found. For hydrocyanic acid this consisted of an increase in the rate and depth of the respiration, while for iodosobenzoate the production of an apnoeic period of 10 to 15 seconds duration was taken as the criterion. After allowing a sufficient time to elapse both substances were injected simultaneously and their effects noted. The experiments showed that there exists a very distinct antagonism between the two substances in their effects on the respiration.

The only objection to this method of determining the antagonism between the substances is that it does not show definitely whether the antagonism is pharmacological or chemical, that is to say, whether they act oppositely upon the respiratory center or mutually destroy one another in the blood stream. It is possible that the substances react in the blood stream, the hydrocyanic acid being oxidized and the iodosobenzoate reduced. We believe, however, that this reaction could hardly be expected to occur so quickly and we regard it as more probable that the antagonism

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is pharmacological. This result then rather corroborates the view that sodium iodosobenzoate increases physiological oxidation. The protocells of two of the experiments showing the antagonism follow. In Experiment II we were fortunate enough to hit upon doses which almost completely neutralized each other.

Experiment I. Male cat, weight 4.3 kilos. Ether anaesthesia

TIME	DRUGS	RESPIRATIONS		BLOOD PRESSURE	PULSE RATE	REMARKS
		Rate	Amplitude			
hr. m.			*mm.*			
4 12½		48	2.5	147	186	
13	1 cc. $\frac{N}{135}$ HCN	56	6.5	154	186	Injection into right femoral.
15½		44	2.5	112	188	
16	1 cc. $\frac{N}{135}$ HCN	44	6.0	120	184	
17½		44	3.5	107	194	
18	2 cc. $\frac{N}{135}$ HCN	64	12.5	115	192	2 cc. of $\frac{N}{135}$ HCN proved to be the threshhold dose for this animal.
22		32	7.5	100	180	Changed respiration adjustment.
22½	1 cc. $\frac{N}{100}$ iodoso	24	9.5	104	180	Injection into left femoral.
24		26	9.5	114	182	
24½	2 cc. $\frac{N}{100}$ iodoso			120	186	9½ seconds after injection there was complete cessation of the respiration lasting 14½ seconds.
25		29	11.0	120	180	
40½		24	8.5	134	184	
41	2 cc. $\frac{N}{135}$ HCN 2 cc. $\frac{N}{100}$ iodoso	25	8.5	134	185	12 seconds after injection complete cessation of respiration for 12 seconds.
45½		26	8.5	104	188	
46	2 cc. $\frac{N}{54}$ HCN 2 cc. $\frac{N}{100}$ iodoso	26	11.0	101	198	15 seconds after injection complete cessation of respiration for 5 seconds. Following this the respiration became very fast and irregular in amplitude.

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Experiment II. Cat, female, weight 3.52 kilos. Ether anaesthesia

TIME	DRUGS	RESPIRATION		BLOOD PRESSURE	PULSE RATE	REMARKS
		Rate	Amplitude			
hrs. *m.*			*mm.*			
10 6½		40	5.5	134	220	
7	2 cc. $\frac{N}{100}$ iodoso	39	5.5	143	228	Injected into left femoral vein.
24½		28	3.0	149	208	
25	2 cc. $\frac{N}{50}$ iodoso	20	3.0	148	208	Apnoea of 6 seconds.
26½		32	3.0	146	220	
27	1 cc. $\frac{N}{20}$ iodoso	12	3.0	152	216	Apnoea of 8 seconds duration.
29		26	3.5	146	220	
29½	1 cc. $\frac{N}{20}$ iodoso			154	212	Apnoea of 18 seconds. See fig. 1.
32		32	4.5	138	224	
32½	2 cc. $\frac{N}{135}$ HCN	36	5.5	139	216	Injected into right femoral vein.
39		32	3.5	148	204	
39½	1 cc. $\frac{N}{54}$ HCN	36	4.5	147	208	
40		29	3.5	147	206	
40½	1 cc. $\frac{N}{54}$ HCN	34	7.0	139	208	
41		30	4.0	136	212	
41½	2 cc. $\frac{N}{54}$ HCN	38	10.0	128	210	Marked dyspnoea. See fig. 2.
47½		24	3.0	134	184	
48	1 cc. $\frac{N}{20}$ iodoso 4 cc. $\frac{N}{54}$ HCN	18	3.0	124	176	There were a few very shallow respirations but neither apnoea nor dysponea developed. Within a half minute the respirations were normal in rate and depth. See fig. 3.

SUMMARY

1. Hydrocyanic acid administered intravenously to rabbits in doses of 0.5 to 1 mg. per kilo or sodium cyanide in doses of 1 to 2 mg. per kilo causes temporary stoppage of the respiration

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in passive expiration for periods varying from a few seconds to nearly four minutes. The injection must be made rapidly.

2. The respiratory center is more sensitive to hydrocyanic acid than the vaso-motor center and the latter is apparently more sensitive than the cardio-inhibitory center.

3. The action of hydrocyanic acid on the respiration and circulation resembles so closely that of certain other drugs which cause temporary stoppage of the respiration (phenylmethyl-isoxazol-chlormethylate; Tappeiner), tetramethyl-ammonium chloride (Jodlbauer), quaternary paverin bases (Pohl) and protocatechyl-tropeine (Marshall) that it suggests the possibility that these drugs owe their activity as does hydrocyanic acid to their power to inhibit physiological oxidation in the cells upon which they act.

4. We have established that a definite antagonism exists between hydrocyanic acid and sodium iodosobenzoate in regard to the respiratory center. This antagonism would further indicate that iodosobenzoate is capable of accelerating normal physiological oxidation.

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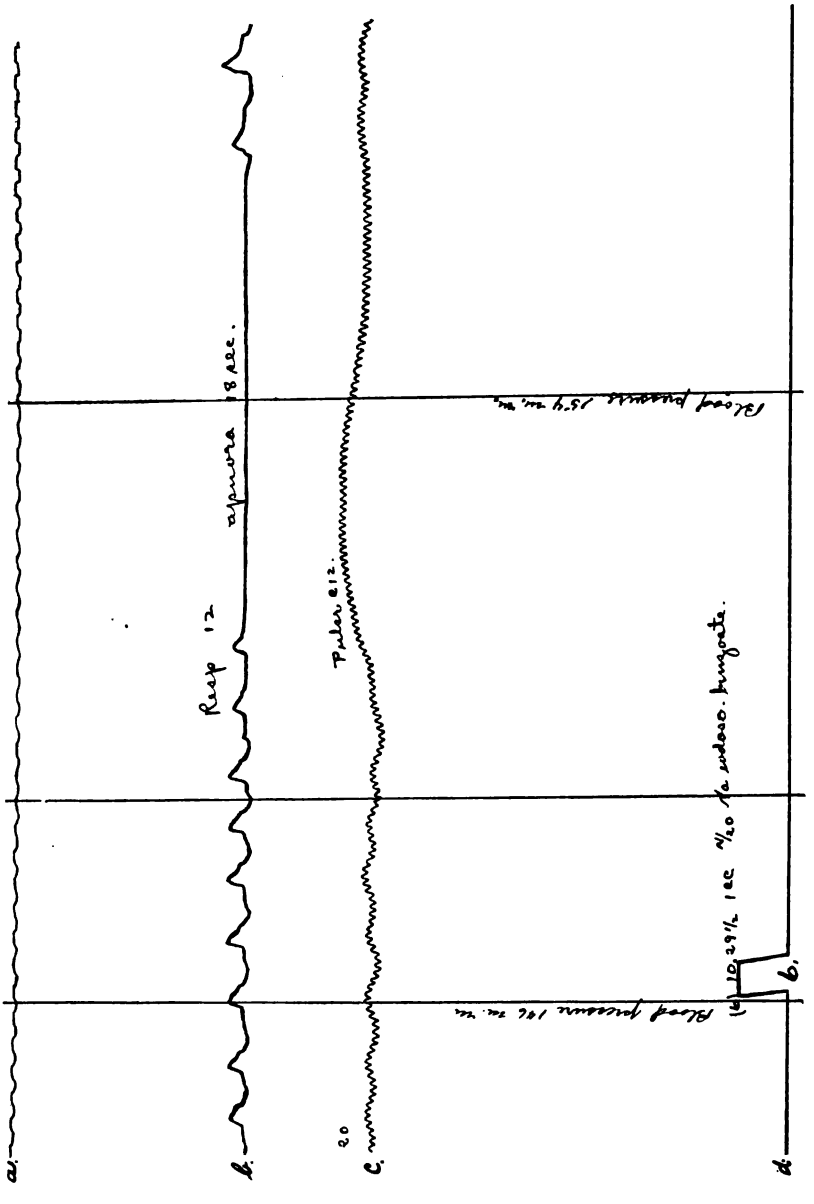


Fig. 1. Female cat, 3.52 K. (See experiment 2.) *a*. Time record in seconds. *b*. Respiratory record from tracheal tube, upstroke expiration. *c*. Blood pressure from Carotid. *d*. Signal pen and zero pressure. At 6, 1 cc. $\frac{N}{10}$ sodium iodosobenzoate injected into left femoral vein. Read from left to right.

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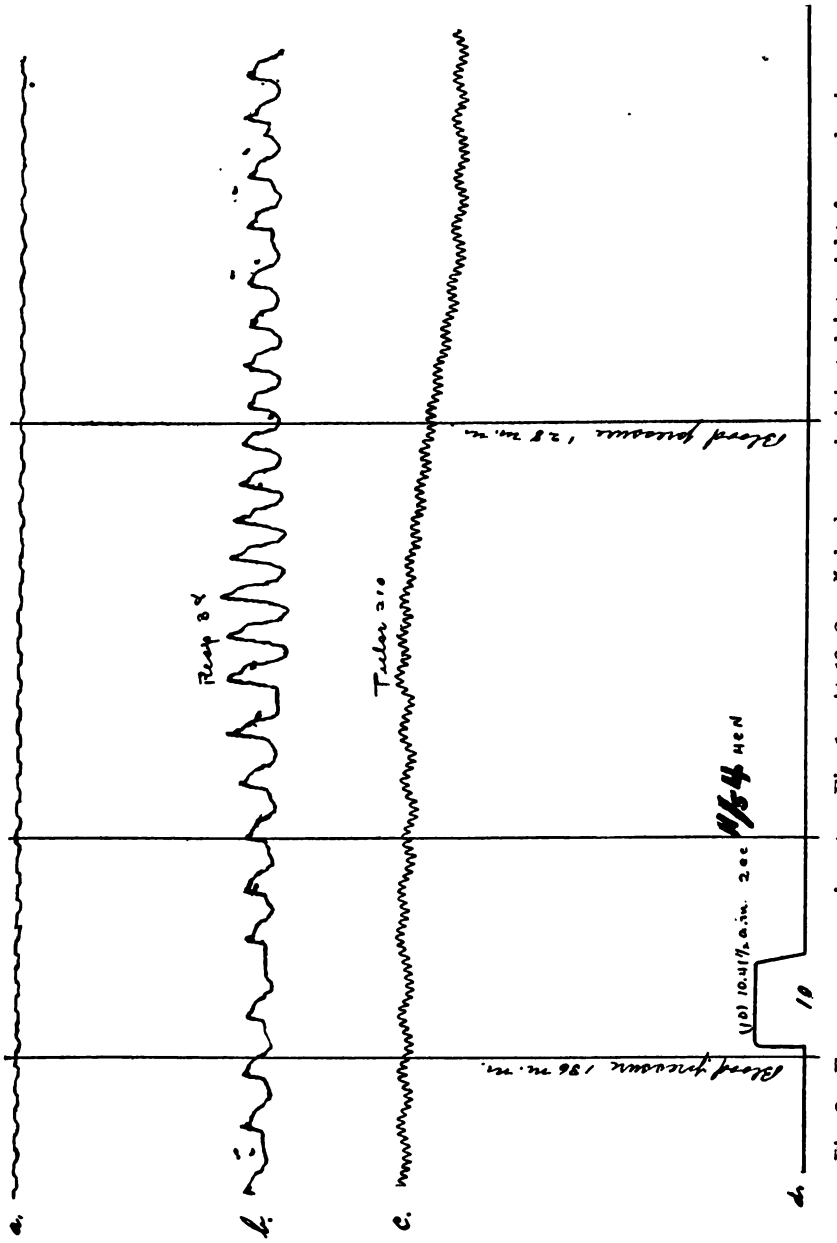


Fig. 2. From same experiment as Fig. 1. At 10, 2.cc.$\frac{N}{54}$ hydrocyanic injected into right femoral vein.

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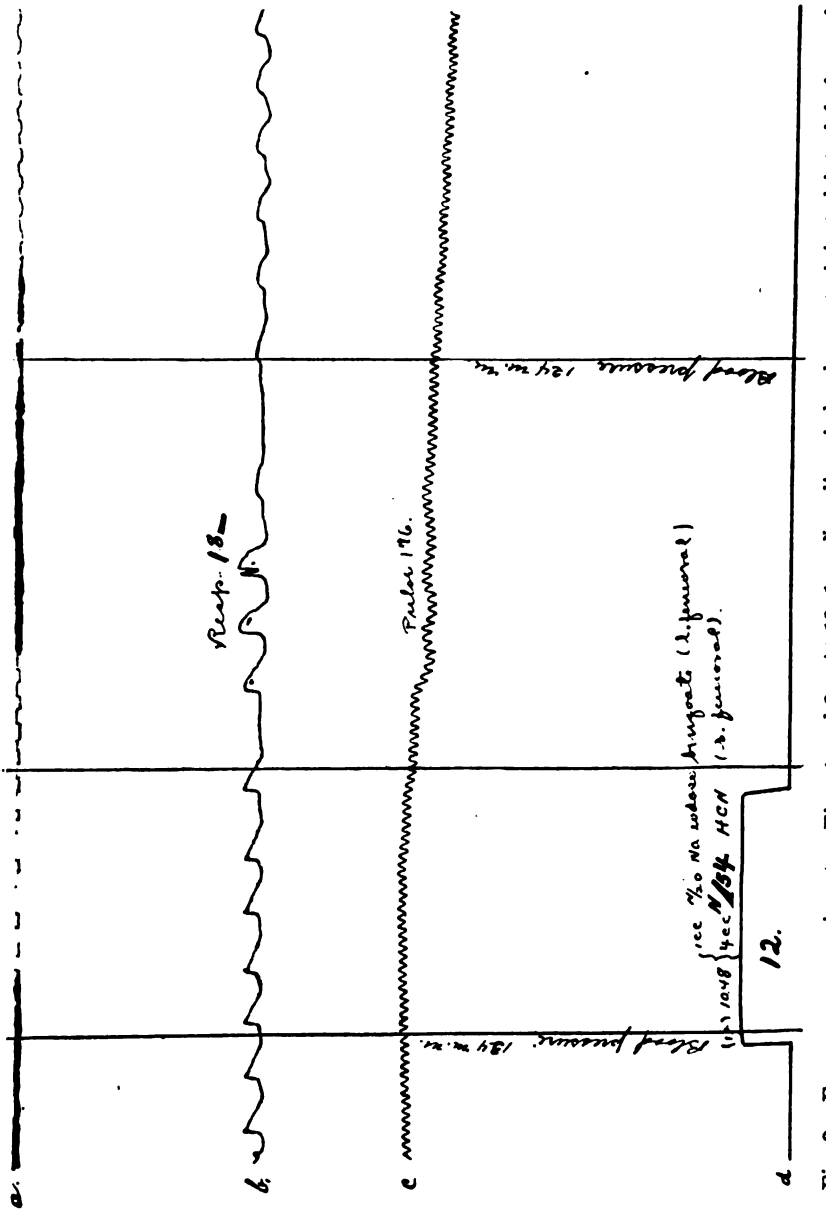


Fig. 3. From same experiment as Figs. 1 and 2. At 12, 1.cc.$\frac{N}{10}$ sodium iodosobenzoate injected into left femoral and 4.cc.$\frac{N}{51}$ hydrocyanic acid injected into right femoral vein.

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ON THE ANTISEPTIC AND BACTERICIDAL ACTION OF THE SODIUM SALTS OF IODBENZOIC, IODOSOBENZOIC AND IODOXYBENZOIC ACIDS[1]

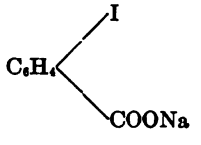
AARON ARKIN

From the Pharmacological Laboratory of the University of Wisconsin

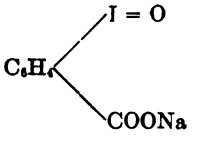
Received for publication, September 2, 1911

It was stated by Loevenhart and Grove[2], that the substances mentioned in the title of this paper were studied in the hope that they would prove useful therapeutically as intravenous antiseptics. Before studying their value as intravenous antiseptics it seemed desirable to ascertain their value as aintseptics in vitro and it is only with this phase of the subject that this communication deals.

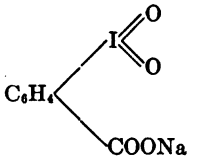
The formulae of these substances are as follows.[3]



Sodium iodbenzoate



Sodium iodosobenzoate



Sodium iodoxybenzoate

It will be noted that the iodbenzoate contains no active oxygen and is not an oxidizing agent. Iodosobenzoate has an atom of very active oxygen connected with the iodine and contains 6.06 per cent of active oxygen. Iodoxybenzoate, with two atoms of active oxygen bound to the iodine, contains 11.43 per cent of active oxygen. A comparison of the antiseptic and bactericidal

[1] The expenses of this research have been partially defrayed by a grant from the Rockefeller Institute for Medical Research.

[2] This Journal (Vol. III, p. 101).

[3] An account of these substances and the method of preparing them has already been presented by Loevenhart and Grove.

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power of these three substances it seemed would throw interesting light on the bactericidal power of oxygen in active form, because the bactericidal power of iodosobenzoate and iodoxybenzoates could differ widely from that of iodbenzoic acid only in consequence of the oxidizing action of the former substances. The reasons for attributing the biological effects of iodosobenzoic and iodoxybenzoic acids, in so far as they differ from the effects of iodbenzoic acid, to the active oxygen, have been discussed by Loevenhart and Grove.

It is especially desired to emphasize the point that we were interested in the bactericidal action of these compounds as active oxidizing agents and not as members of the group of iodine-containing bactericides. Among the most powerful bactericidal substances known are to be found several whose activity depends on their power as oxidizing agents. Among these may be mentioned ozone, benzoyl acetyl peroxide, chlorine, bromine, iodine and the persulphates. Among the less powerful antiseptics of this series may be mentioned hydrogen peroxide and benzoyl peroxide.

It seemed, therefore, that the iodoso- and iodoxybenzoates would prove to possess considerable strength as disinfectants whereas iodbenzoate would be found to be comparatively inert. The experiments proved that this is true.

Methods: In comparing the antiseptic and bactericidal action of substances, it is necessary in order to obtain accurate results to maintain the following conditions: (1) A constant temperature. Throughout this work the organisms were exposed to the bactericides at the body temperature; viz., 37°, because we were interested in these substances as intravenous antiseptics. (2) To use the same strain of organisms throughout the series and to employ as nearly as possible the same number of organisms in each experiment. (3) To employ exactly the same nutrient medium throughout the series. All of the conditions were carefully controlled. A slight modification of the Rideal-Walker[4] method was employed. The organisms studied were B. typhosus, B. coli, B. pyocyaneus and Staphylococcus aureus. The

[4] Rideal and Walker: Jour. Roy. San. Inst., 24, 424. (1903).

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cultures employed were in all cases prepared by inoculating broth tubes from slant agar growths and incubating twenty-four hours at 37°. The tubes were then kept at room temperature until used, which in most cases was not over twelve to sixteen hours.

The bacteria were exposed to the action of the salts in ordinary broth and in the case of B. typhosus and B. coli also in glucose litmus broth (ordinary broth containing 0.1 per cent azolitmin and 1 per cent glucose). In the former case growth was indicated by the tubes becoming turbid and in the latter case by the development of acid which changed the litmus from blue to red. The methods were equally sensitive and equally satisfactory, the results being practically the same in all cases in which they were compared.

In test tubes were placed 8 cc. of broth and 2 cc. of the solution of the salt to be studied. The solution of the salt used was five times as concentrated as it was desired to study. Thus when it was desired to study a salt in $\frac{N}{1000}$ solution, 2 cc. of a $\frac{N}{200}$ were added to 8 cc. of broth. The tubes were inoculated with carefully measured quantities of the broth cultures previously described. In nearly all cases 0.04 cc. of the twenty-four-hour broth culture was placed in the test tube with the disinfectant mixture. The inoculated tubes were then placed in the incubator at 37° for seventy-two hours. This procedure merely determined the antiseptic action of the substances. In order to determine whether the substances had killed the bacteria or had simply prevented their growth, subcultures were made after twenty-four hours incubation from these tubes into fresh litmus or plain broth, using from two to five loopfuls of material. It was shown by separate experiments that the amount of the substances carried over into the second tube was entirely without effect on the growth of bacteria. The tubes from which the subcultures were made were always shaken before inoculating from them. The subcultures were always observed for three days before pronouncing them sterile. We have simply determined, therefore, the concentration of the salts required to prevent growth for three days and also the concentration required to sterilize the tubes in twenty-four hours at 37°.

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Series 1. Sodium iodosobenzoate, B. typhosus, Glucose litmus broth. 0.04 cc. of a twenty-four-hour broth culture was used in inoculating the first set of tubes. Temperature 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Red	A_1	Red
B	$\frac{N}{100}$	Blue	B_1	Blue
C	$\frac{N}{300}$	Blue	C_1	Blue
D	$\frac{N}{1000}$	Blue	D_1	Blue
E	$\frac{N}{3000}$	Red	E_1	Red

This series shows that sodium iodosobenzoate in $\frac{N}{1000}$ solution kills B. typhosus in twenty-four hours at 37°.

Series 2. Sodium iodosobenzoate, B. typhosus, plain broth. 0.04 cc. of a twenty-four-hour broth culture used

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Turbid	A_1	Turbid
B	$\frac{N}{1000}$	Clear	B_1	Clear
C	$\frac{N}{2000}$	Clear	C_1	Clear
D	$\frac{N}{4000}$	Turbid	D_1	Turbid
E	$\frac{N}{8000}$	Turbid	E_1	Turbid

A large number of similar experiments were performed, nearly all agreeing with Series 1 that $\frac{N}{1000}$ iodosobenzoate kills B. typhosus under these conditions.

Series 3. All conditions the same as in Series 1 except that B. coli was used

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Red	A_1	Red
B	$\frac{N}{100}$	Blue	B_1	Blue
C	$\frac{N}{300}$	Blue	C_1	Blue
D	$\frac{N}{1000}$	Blue	D_1	Red
E	$\frac{N}{3000}$	Red	E_1	Red

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In this series B. coli was killed by sodium iodosobenzoate in a concentration between $\frac{N}{800}$ and $\frac{N}{1000}$ in twenty-four hours at 37°. Numerous other experiments showed, however, that B. coli is killed by $\frac{N}{1000}$ iodosobenzoate under conditions as shown in Series 4.

Series 4. Sodium iodosobenzoate, B. coli, plain broth. Condition same as in Series 3

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—THREE LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Turbid	A_1	Turbid
B	$\frac{N}{1000}$	Clear	B_1	Clear
C	$\frac{N}{2000}$	Turbid	C_1	Turbid
D	$\frac{N}{4000}$	Turbid	D_1	Turbid
E	$\frac{N}{8000}$	Turbid	E_1	Turbid

Series 5. Sodium iodosobenzoate, Staphylococcus aureus, plain broth. 0.042 cc. of a twenty-four-hour broth culture used; 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Turbid	A_1	Turbid
B	$\frac{N}{100}$	Clear	B_1	Clear
C	$\frac{N}{300}$	Clear	C_1	Clear
D	$\frac{N}{1000}$	Clear	D_1	Clear
E	$\frac{N}{3000}$	Turbid	E_1	Turbid

This series shows that sodium iodosobenzoate in $\frac{N}{1000}$ solution kills Staphylococcus aureus in twenty-four hours at 37°.

Series 6. Sodium iodosobenzoate, B. pyocyaneus, plain broth. 0.04 cc. of twenty-four-hour broth culture used; 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A	None	Turbid	A_1	Turbid
B	$\frac{N}{100}$	Clear	B_1	Clear
C	$\frac{N}{300}$	Clear	C_1	Clear
D	$\frac{N}{1000}$	Slightly turbid	D_1	Turbid
E	$\frac{N}{3000}$	Turbid	E_1	Turbid

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Hence sodium iodosobenzoate kills B. pyocyaneus in a concentration between $\frac{N}{800}$ and $\frac{N}{1000}$ in twenty-four hours at 37°.

Series 7. A comparison of the germicidal action of the sodium salts of iod-, iodoso-, and iodoxybenzoates on B. typhosus. The organisms were exposed to the antiseptic in plain broth for twenty-four hours at 37° in the usual way at the end of which time subinoculations were made into glucose litmus broth. 0.04 cc. of the broth culture was used in each of the original tubes. The results of the inoculation of the subcultures alone are given below

IODBENZOATE	AFTER SEVENTY-TWO HOURS	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Red	None	Red	None	Red
$\frac{N}{100}$	Red	$\frac{N}{500}$	Blue	$\frac{N}{500}$	Blue
$\frac{N}{200}$	Red	$\frac{N}{1000}$	Blue	$\frac{N}{1000}$	Blue
$\frac{N}{300}$	Red	$\frac{N}{2000}$	Red	$\frac{N}{2000}$	Blue
$\frac{N}{1000}$	Red	$\frac{N}{3000}$	Red	$\frac{N}{4000}$	Red

This series shows that $\frac{N}{100}$ sodium iodbenzoate does not kill B. typhosus in twenty-four hours at 37° whereas the iodosobenzoate kills in $\frac{N}{1000}$ and iodoxybenzoate in $\frac{N}{2000}$ solution. This series of experiments was repeated several times with the same results. Another series of experiments proved that $\frac{N}{10}$ iodbenzoate is required to kill B. typhosus under these conditions.

Series 8. Comparison of iodoso-, and iodoxybenzoates on Staphylococcus aureus. Original tubes and subcultures were made in plain broth. The original tubes received 0.04 cc. of the twenty-four-hour broth culture. Temperature 37°. Observations on the subcultures alone given

IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Turbid	None	Turbid
$\frac{N}{500}$	Clear	$\frac{N}{500}$	Turbid
$\frac{N}{1000}$	Clear	$\frac{N}{1000}$	Turbid
$\frac{N}{2000}$	Turbid	$\frac{N}{2000}$	Turbid
$\frac{N}{3000}$	Turbid	$\frac{N}{3000}$	Turbid

Thus $\frac{N}{1000}$ iodosobenzoate kills Staphylococcus aureus in twenty-four hours at 37° whereas $\frac{N}{500}$ iodoxybenzoate failed to kill them under the same conditions. Further experiments proved that under these conditions $\frac{N}{200}$ iodoxybenzoate was required to kill Staphylococcus aureus.

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Series 9. Comparison of iodoso- and iodoxybenzoates on B. pyocyaneus. Conditions the same as in Series 8. Observations on subcultures alone given.

IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Turbid (green)	None	Turbid (green)
$\frac{N}{200}$	Clear	$\frac{N}{200}$	Clear
$\frac{N}{400}$	Clear	$\frac{N}{400}$	Clear
$\frac{N}{800}$	Clear	$\frac{N}{800}$	Clear
$\frac{N}{1200}$	Turbid	$\frac{N}{1200}$	Clear
$\frac{N}{1600}$	Turbid	$\frac{N}{1600}$	Turbid

This series shows that iodosobenzoate kills B. pyocyaneus in twenty-four hours at 37° in a concentration between $\frac{N}{800}$ and $\frac{N}{1200}$ whereas between $\frac{N}{1200}$ and $\frac{N}{1600}$ iodoxybenzoate kills under these conditions.

These experiments prove that sodium iodbenzoate has very little bactericidal power compared with that of iodoso-, and iodoxybenzoates. Using B. typhosus it was found that the three substances stand in the following relation to one another:

	CONCENTRATION REQUIRED TO KILL	RELATIVE BACTERICIDAL ACTION
Sodium iodbenzoate	$\frac{N}{10}$	1
Sodium iodosobenzoate	$\frac{N}{1000}$	100
Sodium iodoxybenzoate	$\frac{N}{2000}$	200

Thus toward B. typhosus the substance containing one atom of active oxygen is one hundred times as bactericidal as the mother substance containing no active oxygen. The substance containing two atoms of active oxygen is twice as bactericidal as that containing one atom of active oxygen. The results obtained when B. coli was used were the same; i.e., the iodosobenzoate kills in $\frac{N}{1000}$ and iodoxybenzoate in $\frac{N}{2000}$ solutions, but the concentration of iodbenzoic acid required to kill B. coli was not determined. With B. pyocyaneus iodosobenzoate is bactericidal in $\frac{N}{800}$ and iodoxybenzoate in $\frac{N}{1200}$ solution. With Staphylococcus aureus iodosobenzoate kills in $\frac{N}{1000}$ solution whereas iodoxybenzoate

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requires $\frac{N}{200}$ solution to kill under the same conditions. These differences in the relative bactericidal value of iodoso- and iodoxybenzoates towards different organisms are very interesting. It does not in any sense argue against the proposition that the antiseptic action of both is due to their oxidizing action. A great many instances of specificity in oxidations with the simplest chemicals may be pointed out. If A and B each destroy C and D by oxidative processes the fact that C is more sensitive to A than to B does not indicate that D will also be more sensitive to A than to B, indeed the reverse is just as likely to be found true. This statement holds whether C and D are living micro-organisms or relatively simple chemicals.[5]

THE EFFECT OF PROTEINS AND OTHER ORGANIC MATTER ON THE BACTERICIDAL ACTION OF IODOSOBENZOATE AND IODOXYBENZOATE

It is well known that the presence of proteins greatly reduces the efficacy of most bactericidal substances. The reason for this is that most bactericidal substances react with proteins and it is in consequence of this property that they are bactericidal. Hence when the bacteria are suspended in a medium rich in proteins, the bactericidal substance combines more readily with the proteins outside of the bacteria and their action on the bacteria is thereby greatly reduced. Among the bactericidal substances of this group may be mentioned all of the salts of the heavy metals, formaldehyde, and phenol and its derivatives.

[5] An interesting instance of this sort of specificity in oxidative processes was pointed out by Loevenhart and Kastle (Amer. Chem. Jour., 29, 432, 1903). They showed that whereas hydrogen peroxide readily oxidizes formic aldehyde, it is incapable of oxidizing formic acid, yet hydrogen peroxide in the presence of platinum black oxidizes formic acid far more readily than it does formic aldehyde. Similarly formic acid is oxidized by mercuric chloride, whereas formic aldehyde is not and yet formic aldehyde is much more readily oxidized by potassium permanganate in the presence of sulphuric acid than is formic acid. These factors seem not to have been sufficiently considered by Cushny, in his work "On the action of oxidizing salts" Arch. f. exper. Path. u. Pharm. Schmiedeberg-Festschrift, 1908, p. 126.

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The work of Ehrlich and Bechhold[6] brought out beautifully the effect of proteins in protecting bacteria from the action of a bactericide whose action in all probability depends on its power to combine with proteins. Ehrlich and Bechhold found that tetrachlor-o-biphenol prevents the growth of B. diphtheriae in a dilution of 1:320,000 in the absence of protein, but in the presence of blood its antiseptic action was so reduced that a concentration of 1:10,000 did not entirely prevent growth. Bechhold[7] succeeded in proving, by means of his gelatin filters, that his substance was combined with the proteids of the blood.

Since proteins do not readily suffer oxidation, and since our substances owe their bactericidal action to their oxidizing power, we were led to believe that proteins would not greatly influence the bactericidal action of iodoso- and iodoxybenzoate. This was found to be the case.

Series 10. About 300 cc. of blood were drawn aseptically from a large dog. This was kept for forty-eight hours at room temperature, when the serum was pipetted off and placed in the ice chest for twenty-four hours. The serum was heated to 55° for one hour in order to destroy its bactercidal action. The serum was straw colored and contained no haemoglobin. Each tube contained 5 cc. of serum and 1 cc. of iodoxybenzoate of proper strength to give the concentration stated in the table. Each tube was inoculated with 0.0194 cc. of a broth culture of B. typhosus. The subinoculations recorded in the table were made into glucose litmus broth after the original tubes had been incubated twenty-four hours at 37°

	IODOXYBENZOATE	SUBCULTURES AFTER SEVENTY-TWO HOURS
A	None	Red
B	$\frac{N}{500}$	Blue
C	$\frac{N}{1250}$	Blue
D	$\frac{N}{2400}$	Blue
E	$\frac{N}{4800}$	Red
F	$\frac{N}{9600}$	Red

[6] Zeitsch. f. physiol. Chem., 47, 173 (1906).
[7] Zeitsch. f. physiol. Chem., 52, 177 (1907).

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A similar experiment with unheated serum gave the same result.

Series 11. This series of experiments was carried out as described under Series 10 except that a 5 per cent gelatin solution was substituted for the serum

	IODOXYBENZOATE	SUBCULTURES IN GLUCOSE LITMUS BROTH AFTER SEVENTY-TWO HOURS
A	None	Red
B	$\frac{N}{200}$	Blue
C	$\frac{N}{400}$	Blue
D	$\frac{N}{1000}$	Blue
E	$\frac{N}{2000}$	Blue
F	$\frac{N}{4000}$	Blue

These experiments show that the bactericidal action of sodium iodoxybenzoate is not diminished either by the presence of $83\frac{1}{3}$ per cent dog serum or $4\frac{1}{6}$ per cent gelatin.

SUMMARY

1. Sodium iodoso- and iodoxybenzoates have far greater bactericidal power than sodium iodbenzoate. Toward B. typhosus sodium iodosobenzoate is one hundred times and sodium iodoxybenzoate is two hundred times as bactericidal as sodium iodbenzoate. This difference must be attributed to the oxidizing action of the former substances.

2. Toward B. typhosus and B. coli sodium iodoxybenzoate is twice as strong as sodium iodosobenzoate.

3. Toward B. pyocyaneus sodium iodoxybenzoate is about 1.5 times as bactericidal as sodium iodosobenzoate.

4. Toward Staphylococcus aureus sodium iodosobenzoate is about five times as bactericidal as sodium iodoxybenzoate.

5. The presence of either $83\frac{1}{3}$ per cent blood serum or $4\frac{1}{6}$ per cent gelatin does not diminish the bactericidal action of sodium iodoxybenzoate.

6. The comparison of the bactericidal action of these three subtances furnishes an interesting example of the influences of chemical constitution on bactericidal action.

In conclusion I desire to thank Dr. A. S. Loevenhart for suggesting this work to me and for his assistance in carrying it out.

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THE BIOLOGICAL BASIS OF SPECIFIC THERAPY.*

BY SIMON FLEXNER, M.D., D.SC.,

Director of the Laboratories of the Rockefeller Institute for Medical Research, New York.

I DESIRE to express to the Trustees of this splendid hospital the sense of the honor conferred in the invitation to address you on this historic occasion. Since no pen can portray the horrors of surgery in pre-anesthetic days, so no words can fitly describe the benefits that have flowed from the demonstration of surgical anesthesia in this institution sixty-five years ago. We may, however, unite in reverence to the memory of the wise and heroic men who participated in that famous event.

Remarkable achievements are never unique occurrences in nature. Even the greatest men rest on the shoulders of a large multitude of smaller ones that have preceded them; and epochal discoveries emerge out of a period of intellectual restlessness that affects many minds. And thus the decade between 1840 and 1850 was not barren, even in medical annals, of portending events other than the discovery of anesthesia. It was the period of publication of Henle's prescient essay, "Von den Miasmen und Kontagien," that forecast with astounding accuracy of detail the foundations of the parasitic origin of disease; and of the observation by Cornalia of the corpuscles in the silkworm, which a decade later, in Pasteur's magical hands, were to become the instrument through which

*Address delivered at the Massachusetts General Hospital on the sixty-fifth anniversary of Ether Day, Oct. 16, 1911.

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the medical profession and the world generally were about to be aroused to the significance of germ life as causes of diseases of the higher animals. And since then, by leaps and bounds, and through a process of logical evolution, a multitude of important diseases have been traced to the specific micro-organisms that cause them, and the complex biological conditions that determine and then suffice to defeat the ultimate consequences of infection have been gradually but surely unfolded and explained. I have chosen the theme, "The Biological Basis of Specific Therapy," to present before you because it seemed fitting to commemorate the occasion by emphasising another great achievement in the conquest of disease, and because my own interests and studies have carried me into this particular field of investigation.

In the pursuit of knowledge of this subject no aspect of the problem has been more enlightening and rewarding than that relating to the reasons for spontaneous recovery from infectious disease. The leading physicians have rarely failed to appreciate the unexcelled power of "Nature" herself to heal her self-inflicted wounds and to recognize that many diseases tend of themselves, when not quickly fatal, to progress toward recovery. There resides, therefore, within the animal body a set of potential forces capable, when aroused, of exercising a highly effective control over disease. Many of you are familiar with the fact that these powers have been traced to a group of substances contained within the blood and passing from the blood into the lymph, where they exert influence on the cells composing the organs and upon parasites in the interstices of the tissues.[1] What these substances consist of has also been ascertained in good part, so that

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they may be classed briefly into soluble, complex chemical bodies, probably of protein nature, that are contained dissolved in the fluids, and of certain motile white cells, the so-called leucocytes or phagocytes. In virtue of the soluble form and the motility of the cells, these healing substances are able to reach most parts of the body where their special properties may be exerted. Moreover, not only are these curative substances, technically called "immunity principles," preformed in all individuals in which they operate against intending infections, but they become quickly increased in amount when infection has been established; and the ultimate issue of the condition in spontaneous recovery or the reverse depends upon the degree of this response to infection and the competency of the curative principles evoked to reach and to suppress the infectious agents.

These principles come to operate equally

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[1] The native curative powers of the blood have been invoked to heal local diseases through the creation of a condition of artificial hyperemia or congestion. "All organs that functionate are hyperemic during activity. In every form of growth and regeneration local hyperemia is present and in a degree corresponding to the rapidity and energy of the growth. . . . All reactions to foreign substances, whether crude bodies or minute parasites or their chemical products, are associated with hyperemia. There is no lesion which the body tries to and is capable of removing by rendering harmless that produces anemia. Hence, if we accept the reactions of the body as useful efforts of nature, we must admit that hyperemia is the most common of all auto-curative agents." (Bier: Hyperemia. Translation by G. A. Blech.)

[2] A number of diseases of the higher animals, including man, and one disease of plants (the mosaic disease of tobacco) have, within ten years, been traced to sub-microscopic parasites. It is indeed not remarkable that the present microscopes should have failed to define the limits of organized nature. Whether we shall ever invent instruments capable of resolving and rendering visible these minute particles of living matter is a question impossible to answer. Even doubling the potential power of the microscope by the device of employing, for photographic purposes, the ultra-violet rays of the spectrum has failed to bring them into view. Their place in nature is not accurately established. Some, as the parasite causing yellow fever, that passes a stage of its existence in mosquitoes, probably are protozoal; others, as the parasite of pleuropneumonia of cattle, that can be propagated in artificial cultures, probably are bacterial. It can hardly be doubted that they are living organisms since they are capable of transmission from animal to animal, in which they produce infection, through an indefinite series.

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against all classes of microbic parasites, whether protozoa, bacteria or that remarkable class the import of which we are just learning — the so-called submicroscopic or filterable organisms or viruses.[2] But the effectiveness of the operation is determined not only by the intrinsic qualities of parasite and of host, but also in a high degree by the manner of location and distribution of the parasites themselves within the infected host. Whether they have a general distribution throughout the blood and tissues, or whether they are confined within a pathological process in the interior of an important organ or part, may be the factor determining whether not only the native curative principles shall gain ready access to them, but whether, also, extraneous curative agents introduced into the body shall be able to reach the seat of disease.

The parasite, struggling to survive, withdraws, at one time, into situations to which the curative substances gain access imperfectly and with difficulty, causing thereby local infections more or less cut off from the general circulation and the curative substances purveyed by the blood. This is the condition met with in massive inflammations, in abscess formation and in infections of specialized portions of the body — such as the great serous cavities — that receive normally a modified and dilute lymph-secretion.

It is the lymph which carries the protective, as it does the nutritive, principles for the tissues and organs; and hence this fluid provides the essential safeguard against infection. Moreover, the quality of lymph in the several serous cavities is not the same, but is indeed peculiar for each cavity, and the lowest limit of strength is reached by the cerebrospinal fluid, — regarded as the lymph of the brain and spinal cord, — which

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is almost devoid of protein matter.[3] As the protein moiety of the lymph carries the immunity principles, it follows that the serous cavities are, really, less well supplied with them, and the subarachnoid spaces of the central nervous system the least well of all. These considerations are not without high importance as affecting the perfection of the provisions for warding off, or, later, for controlling and abating, an infection. Since the anatomical structure decides the quality of the lymphatic fluid in health, it also determines it in disease, and thus by regulating the composition of the fluid commands the issue of the pathological process. Under such circumstances the parasites that become localized in these cavities are insured a potential advantage against the host.

The parasites possess, moreover, an advantage of regulation within themselves to preserve them from extinction — they are capable of altering rapidly, not their form and external appearances, but their chemical reactions and probably structure when too closely pressed and menaced. The change consists in the development of a state of effective resistance — called "fastness" — to injurious chemical agents, whether the immunity principles of the blood or other substances. The new qualities acquired have been viewed as the result of mutations among the

[3] The notion that the cerebrospinal fluid is the lymph of the central nervous system is open to discussion. Mott (*The Lancet*, 1910) suggests that it "may serve as the ambient fluid of the neurons and play the part of lymph to the central nervous system." The fluid arises from the choroid plexus and escaping from the foramina of Magendie and Luschka into the subarachnoid spaces occupies them all and communicates, probably, with a "canalicular system surrounding the cells and vessels of the brain." (Mott.) Thus this fluid should provide the most direct path for the penetration of active substances to the nervous tissues; and, in fact, it has been established by experiment that chemical bodies act upon the nerve cells with greater energy and certainty when introduced directly into the cerebrospinal fluid. The hen, indeed, is not subject to the effects of tetanus toxin injected into the blood, while it suffers from tetanus when the toxin is injected into the subarachnoid spaces. (Behring.)

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parasites, and the mutants have been observed to transmit the new characters through an indefinite number of generations. It is precisely this property of mutation that we are learning to hold accountable for the troublesome or dangerous relapses that occur in many of the parasitic diseases — commonly in, for example, malaria, sleeping sickness, spirochetal infection, to mention only a few.[4] Finally, the so-called chronic carrier of infectious organisms, who is being recognized as a serious menace to the health of society and is sincerely to be pitied, is to be regarded often as the victim of this form of mutation among the micro-organisms, which at one time caused him to be ill, but to which he but not his fellows has become adapted. In the successful exploitation of specific therapeutic measures account must obviously be taken of the biological conditions described, as well as others that may in time be discovered.

Manifestly, therefore, the bringing of the parasitic causes of microbic diseases under the influence of curative agents will be more readily and certainly accomplished when they are widely disseminated throughout the body than when they are hidden away within an organ or in the interior of a serous cavity. Hitherto the most effective agents of specific treatment have been just those that operated against the generalized infections, of which examples are such drugs as quinine in its action against the malarial parasite and mercury in its effect on the spirochetal cause

[4] This parasitic mutation, or "fastness," is more readily developed against serum immunity principles (antibodies) than against chemical agents of the nature of drugs, but once produced the latter effect is the more difficult to remove. Serum fastness may be overcome by the superinfection of an animal that has recovered from infection with the corresponding "fast" strain, through which reversion to the normal type may be accomplished; while chemical mutation is overcome solely, apparently, through sexual conjugation of protozoal parasites in the body of an appropriate insect host (Ehrlich: Folia Serologica, 1911, p. 697).

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of lues. The same result is now being achieved by salvarsan, recently perfected by Ehrlich, in respect to its application to a number of spirochetal affections in man and the domestic animals; while the control of diphtheria by antitoxin, perhaps the most perfect example of all, consists essentially in the neutralization of an universally distributed toxic or poisonous agent that is directly the source of the serious effects of the disease. When in generalized infections the surviving micro-organisms escape from the blood and tissue, as sometimes happens in luetic or other diseases, to aggregate in special situations and local pathological products that are reached imperfectly by the lymph, then the specific drug or other agents assert their curative power with far more difficulty and far less certainty.

Medicine is now armed with a number of specific remedies for serious diseases, consisting partly of chemical compounds of known composition and partly of more complex serum products of unascertained nature. The number of drugs is potentially greater than the number of sera and is capable of almost unlimited expansion, so that doubtless therapeutics will be greatly enriched by future discovery in this fascinating field. That many immune sera are capable of being prepared artificially is also certain, but the degree of their applicability will need to be worked out in any given instance. It is already clear that the immune sera closely resemble the natural defenses against infection and its consequences, so that it follows that they are essentially non-foreign bodies, and thus, technically, ideal agents with which to combat disease. They are, in essence, so precisely fashioned as to operate exclusively against the agents of infection and thus to pass over without molestation the sensitive cells of

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the organs. In fact, their action is less specific than this statement implies because as now manufactured they carry with them, in the natural serum of animals, certain alien substances that do affect, in some degree, the host himself.

A factor that bears upon the production of curative immune sera as well as upon specific drugs is that of fastness or mutation of the microorganisms within the body. Experiment has already disclosed the high importance of this unexpected phenomenon of infection. In the choice of especially fashioned drugs the two properties that now determine availability for practical medical employment are, first, a low degree of toxicity for the organs of the host, and, second, absence of tendency to produce "fast" strains of the parasite upon which they exert their influence.

We have still to learn the extent to which specific drug treatment of the infections is capable of altering the state of the acquired immunity to infectious diseases that protects, in some instances, from second infection or attack of a malady. Important data bearing on this subject are already appearing in connection with the more energetic modes of treatment recently introduced for the spirochetal infections. It now seems that possibly the refractory state in these infections is the result of an enduring subinfection, the complete removal of which exposes the individual to reinfection.[5] In a similar manner it

[5] Ehrlich (*loc. cit.*) explains this phenomenon in a slightly, but not fundamentally, different manner. He accounts for the decreasing number of spirochetæ, as the disease advances, by a wiping out of the parasites through the action of the successive specific antibodies formed. The fresh outbreaks, or relapses, then, are caused by mutants or fast strains that are immune to the antibodies thus far elaborated, and the ultimate subsidence of the lesions depends on the production of antibodies for all the new strains. During the actual existence of the syphilitic infection, insusceptibility to reinfection is secured by the presence of antibodies in the blood to which the strain of spirochetæ, intending to infect, is not immune. But once the disease is actually terminated and all the antibodies have been discharged, reinfection with a normal strain becomes possible.

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would appear that in the suppression of microbic agents of disease by the body's forces through a process of active immunization the serum products are more varied and complex than are produced in the course of artificial immunization of animals that are destined to yield sera to be employed passively, by injection, in the treatment of their corresponding diseases; and that this greater complexity arises from the circumstance that in the suppression of the micro-organisms in the infected body, not only the normal strains, but also the mutants, are successively overcome, with the result that a series of immune principles, each directed against its particular variety of parasite, is elaborated. Diseases of a relapsing character are accounted for on the basis of the conception that each successive relapse coincides with the appearance of a new mutant of the infecting organism; and the most typical disease of this class — relapsing-fever, so-called — is characterized by the ability of its spirochetal cause to undergo at most threee or four mutations, that in turn lead to an equal number of relapses, that, if survived, are followed by an enduring disappearance of the infection. Hence in the artificial production of curative sera we shall have to take account of the mutants or " fast " strains of the micro-organisms used for immunization purposes. This result is not necessarily accomplished, although it may be promoted, by selecting cultures from many different sources. What is required is that we shall learn to distinguish the " fast " strains or mutants outside the body in cultures, and even, indeed, to create them at will so that they may be employed for enriching the sera produced in animals, that will thus be better adapted to their purpose of suppressing the parasitic causes of disease.

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The successful issue of specific therapeutics, towards which goal our hopes have been eagerly turned by the triumphs of experimental medicine, will be secured not only by the elaboration of more perfect instruments for the suppression of the microbic causes of disease, but also through a more effective application of the curative agents themselves to the seat of disease.

I have alluded to the circumstance that the infectious agent may be strengthened in its attack by confinement within the organism, through which confinement it is preserved from injury by the defensive principles in the blood and lymph. Now no group of infections is in position better to secure this protection than that located within the membranes surrounding the brain and spinal cord, the lymphatic contents of which are so poor in defensive principles. And for this reason, and for the reason also that the lymphatic spaces in these membranes are in such intimate association with the pericellular spaces about the sensitive nerve cells, the consequences of meningeal infections are highly serious. To endeavor to reach the infections seated in the membranes by means of the general blood and lymph circulation is futile because of the established fact that not only are the large protein molecules, which include the immunity principles, not secreted within the membranes, but also because highly diffusible salts tend as well to be excluded.

But what cannot be thus accomplished by indirection can happily in this important instance be achieved by direction. No operation is simpler in competent hands than lumbar puncture, so-called, which came into use originally to provide cerebrospinal fluid for purposes of diagnosis and now promises to be of far greater value in affording the means of local specific treatment of menin-

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geal affections. How valuable this route may be for the introduction of curative agents is illustrated at the moment best, perhaps, by the convincing results that have been obtained in the treatment of epidemic cerebrospinal meningitis by the anti-meningitis serum. This therapeutic agent is utterly without effect on the local infection when introduced directly or indirectly into the blood, but it has proven of unmistakable value when injected into the seat of the disease by lumbar puncture. The latest figures relating to its employment are, as should be, the most favorable to its action, since the method of production and administration has been improved through experience. And, therefore, it is a subject of gratification that in the recent French epidemic of meningitis the gross mortality among cases treated by serum injections, begun within the first three days of illness, fell below 10%.

The results secured in epidemic meningitis have suggested the extension of the method of direct local specific treatment to still other kinds of infection of the meninges. Meningitis is now known to be caused by a number of micro-organisms, including the streptococcus, staphylococcus, pneumococcus, the bacillus of tuberculosis and of influenza. Generally speaking, all these inflammations are highly fatal in character. There is still doubt whether recovery from tuberculous meningitis ever takes place; the number of recoveries from pneumococcus meningitis is surely very few; and while we are just learning the extent to which influenzal meningitis prevails, we can already predict that the infection is not only not infrequent, but is highly fatal in character.

Many cultures of influenzal bacilli have slight or no appreciable action on animals and cannot,

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therefore, be employed for purposes of artificial immunization. But cultures obtained from cases of influenzal meningitis not only can be used for preparing an immune serum, but produce, also, when injected into monkeys, a form of meningitis that in its nature, course and fatal effects cannot be distinguished from the spontaneous human affection. This experimental fatal disease, like epidemic meningitis, can be favorably influenced and even controlled by the intraspinal injection of an anti-influenzal serum. The degree of applicability of this serum to the treatment of the spontaneous disease in human beings is still to be determined; but in view of its highly fatal character, it should be tried. Undoubtedly, it will be necessary to apply the serum early and by repeated injections to secure beneficial results, — and the early application will be dependent upon prompt bacteriological diagnosis, which can be made by immediate bacteriological examination of the cerebrospinal fluid.[6]

Influenzal meningitis as it occurs spontaneously or is produced experimentally is attended by an an invasion of the blood with the influenzal bacilli that sometimes appear there in large numbers. It is important, therefore, to consider the consequences of the bacteremia, as it is called, upon the local treatment of the meningeal infection. Now, fortunately, the difficulties surrounding the passage of the antiserum from the blood into the cerebrospinal fluid are directly contrasted with the ease with which the antiserum escapes from the meninges into the blood. This discrepancy is explained by the fact that while the fluid on entry is in the nature of a secretion from the choroid plexus, the escape is by way of the veins in the membranes themselves.

[6] See Wollstein: Jour. Exp. Med., 1911, xiv, p. 73.

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While, therefore, it is impracticable to bring the antiserum into the meninges from the blood, the reverse effect is readily accomplished. And thus it is seen that in such secondary infections of the circulation with bacteria as are being here considered, the suppression of the local process not only stops the eruption of bacilli which causes the blood infection, but the passage of the antiserum from the membranes into the blood arrests their development there.

Probably recovery from any local bacterial infection is not wholly accounted for by the several activities of blood serum and phagocytes that are usually invoked to account for the phenomenon. This restricted view leaves out of consideration certain definite chemical substances that are always present in a focus in which tissues and cells are disintegrating. That some of these are injurious to bacteria, we now know. The nature of these so-called stable bacterial substances yielded by extraction of somatic cells is still doubtful, but among them would appear to be certain soaps yielded by disintegration of the neutral and higher phosphorized fats. That soaps are injurious to bacteria has been abundantly proven, so that the view should be entertained that the degeneration of leucocytes and tissues that results from a local bacterial infection may not be entirely to the advantage of the parasitic agent, but is also of use to the body in assisting it to overcome the organisms, since the cells brought to death and disintegration by the parasites yield chemical substances that themselves exert a destructive action upon the infecting bacteria.

The application of these considerations to the treatment of a typical pneumococcus infection such as the experimentally produced pneumococ-

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cus meningitis in the monkey has been rewarded with significant results. We are still ill-informed of the factors which control resistance to and recovery from a local pneumococcus infection. The decrease in number of these organisms that takes place as recovery progresses — as in lobar pneumonia — has not been shown to depend either on phagocytosis or on serum solution of the bacteria. It is a highly suggestive fact that the pneumococcus differs from most bacteria by reason of its solubility in chemical solutions, such as those containing bile-acids, and, as has recently been discovered, soap. The effect of soap is peculiar in that exposure of the pneumococci to its weak action merely modifies their texture, without altering their capability of growing in cultures, so that, when they are next exposed to blood serum, and especially to an anti-pneumococcus serum, they suffer complete solution. These conditions are, indeed, present in a local pneumococcus infection, since soaps are produced there, and during its progress immunity principles appear in the blood and lymph.[7]

By employing a suitable combination of sodium oleate and anti-pneumococcus serum, experimental pneumococcus infections of the meninges can be controlled and abolished. Through this means monkeys that would surely have succumbed have been repeatedly restored to health. But the successful employment of the soap and serum mixture rests upon the overcoming of the property which the soap possesses of uniting with the protein of the antiserum and of thus being rendered inert and withheld from acting upon the pneumococcus. This obstacle is the common one on which so many high hopes of the chemical suppression of infections by what is

[7] See Lamar: Jour. Exp. Med., 1911, xiii, p. 1.

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termed " internal antisepsis " have been wrecked. Luckily in this instance it has been proved that the soap portion can be kept apart from the protein moiety of the serum by introducing a second protective chemical body, itself innocuous, into the mixture. When minute quantities of boric acid are thus introduced the soap is isolated and left in condition to exert its injurious action upon the pneumococci, for which it appears to have a greater affinity than for ordinary protein matter.[8]

The antisera and the chemical disintegration products of cells do not exhaust the list of natural defensive agents that operate against infection, for there remain the living leucocytes themselves. Certain bacterial infections having not been made to respond to the dissolved immunity substances may still be open to influence by the white blood cells. The effort has been made, with an encouraging degree of success, to control experimentally produced tuberculous pleurisy in the dog by the repeated injection of living leucocytes[9]; and these observations are now being extended to experimental tubercular meningitis, also in the dog, the course of which it has been found possible to influence in a favorable manner.[10] And in these conditions, as in those of influenzal bacillus and pneumococcus meningitis, the blood and general organic infections have been suppressed

[8] Whether among the products of local tissue disintegration a similar separation of the soap and serum elements is secured has not been ascertained; but we should consider factors that possibly suffice to overcome this initial impediment to the bactericidal action of the soaps, among which are the proximity of the bacteria to the nascent fatty acids and soaps and the natural occurrence within the exudate of chemical bodies that have the effect of removing the protein inhibition. The fatty acids and soaps are yielded by the dissolution of the neutral and the higher phosphorised fats contained within the cellular protoplasm in which other colloidal bodies of a protecting nature may well be stored.

[9] See Opie: Jour. Exp. Med., 1908, x, p. 419.

[10] Manwaring: Unpublished researches.

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or reduced with the control of the local disease in the meninges.

While the subject of the treatment of these tuberculous affections with leucocytes is still in the experimental stage and is not ready for application to medical practice, it has been described in this connection in order that there should be brought under review the diverse means that are invokable in the effort to determine the conditions that underlie the therapeutic control of varied infectious processes.

Finally, the principle of the local treatment of infections holds out hope of some measure of therapeutic control, at least, of that serious and menacing disease, now in the foreground of interest for physicians and public alike, namely, epidemic poliomyelitis. The propagation of the disease in monkeys has led to the elucidation of its cause and pathology while at the same time it has led to therapeutic experimentation. The cause of the malady is an exceedingly minute parasite — submicroscopic and filterable — that gains access to the spinal cord and brain by way of the meninges and probably through the perineural lymphatics of the olfactory filaments (nerves of smell) terminating in the nasal mucosa, which are in direct communication with the subarachnoid spaces. The lesions of the meninges are very important in this affection and especially of those prolongations about the veins and arteries entering the spinal cord and bulb, that support the perivascular lymphatics. The lymphatics, and, indeed, the subarachnoid spaces in general, comprise a system of communicating channels, charged with cerebrospinal fluid, that penetrate to the nerve cells and include the pericellular spaces. Consequently a parasitic or toxic agent that gains access to the cerebrospinal fluid

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is capable of ready transportation to all parts of the nervous system, and by the same route it is obviously possible to distribute what may prove to be a soluble antagonistic and therapeutic agent.

Recent experiments have shown unmistakably that spontaneous recovery from poliomyelitis is brought about by a set of usual immunity reactions that involve the formation of soluble principles or antibodies in the blood for the parasitic virus. Similar principles are formed in monkeys; and they can be used successfully up to a certain point when injected into the spinal canal by lumbar puncture in preventing the development, after an intracerebral inoculation of the virus, of experimental poliomyelitis. This effect has not yet been accomplished by the introduction of large quantities of so-called "immune blood" into the circulation, a result that was predictable in view of the location of the pathological process in the meninges that leads to paralysis.

It is not impossible that epidemic poliomyelitis may be favorably affected therapeutically by drugs. There is one drug — urotropin or hexamethylenamin — that does exert some action even when administered by the mouth since it presents the exceptional instance of a chemical body being excreted into the cerebrospinal fluid.[11] But its powers are limited. However, the structure of the drug is one that permits of so many modifications of its composition without the sacrifice of its central structure through which formaldehyde may be liberated, that it has been found readily possible to prepare a number of derivatives, far exceeding urotropin in activity, some of which have been applied to the treatment of experi-

[11] See Crowe: Bull. Johns Hopkins Hosp., 1909, xx, 102.

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mental poliomyelitis, and with a hopeful measure of success. These new compounds, however, require to be injected into the spinal membranes and act best in conjunction with an immune serum.[12] This is obviously merely a beginning in the effort to accomplish the therapeutic control of this protean and highly serious disease, the natural history and significance of which are just beginning to be appreciated. But the outlook for its conquest is at the moment made hopeful through the utilization of the method of local specific treatment by means of which the curative agents can be applied directly to the seat of the disease.

. . . . . .

To have conquered pain through anesthesia, to have abolished wound infection by asepticism, to have accomplished already the prevention of some serious diseases and to have brought the prevention of others within measurable distance, to have learned that many of the organic diseases of past middle life originate in an infection and may thus be prevented, and to have established the guiding principles of the specific treatment of the infectious diseases, are some of the achievements in medical science during the sixty years that have elapsed since the historic demonstration of surgical anesthesia in this venerable institution.

[12] The advantage to be secured against the parasites by employing more than one antagonistic agent results, first, from the circumstance that an antibody or drug will operate with greater effect against an already injured than against a normal parasite, and, second, because mutation in two directions is less readily effected than in one direction. Hence a fortunate combination of serum antibodies and a drug offers, theoretically, a favorable means of overcoming an infecting microorganism. Ehrlich (*loc. cit.*) recommends the simultaneous employment of two curative substances, one of which is especially chosen so as to injure the protoplasm, and the other the nuclei of the parasites.

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ON THE NATURE AND SEAT OF THE ELECTROMOTIVE FORCES MANIFESTED BY LIVING ORGANS[1]

1. When an organ of an animal or a plant is injured an electromotive force develops between the injured and the non-injured surface, the latter being, as a rule (if not always), positive to the former. Loeb suggested in 1897 that this difference of potential might be due to the fact that the injured spot formed an acid and that on account of the H-ion moving faster than the anion a difference of potential was produced. This assumption accounted for the sense of the E.M.F. in a partially injured organ. It was, however, pointed out that the order of magnitude of such diffusion elements is too small to account for the E.M.F. found in living organs. Wilhelm Ostwald had suggested the possibility that living organs form concentration elements with a solid phase interposed, the solid phase —the membrane—being permeable for certain ions only. Bernstein starting from Helmholtz's conception of free energy conceived the idea that measurements of the effect of temperature on the E.M.F. of a partially injured muscle or nerve might indicate the nature of the elements to which these systems belong. He reached the conclusion that the partially injured muscle belongs to the type of concentration element in which a solid phase—the membrane—separates the two liquids, the solid phase being only or more permeable to kations than to anions, thus corroborating Ostwald's suggestion.

Bernstein found that the E.M.F. of the muscle rises in general with the temperature and that it approaches a value in proportion to the temperature. The agreement was, however, not as good as should be desired to put the theory of concentration cell of the muscle current on an absolutely safe basis. Moreover, experiments on nerve were less satisfactory and in both cases accessory assumptions were required to make the actual results agree with the theory.

2. Muscles and nerves are, perhaps, too variable or rather perishable to investigate quantitatively with any degree of satisfaction the nature and origin of their E.M.F. We selected for this purpose a hardier and more constant object, namely, apples, the surface film of which is strong and which remains sufficiently constant during such an investigation. Instead of testing the effect of temperature on the E.M.F., we selected the effect of the concentration of the solutions in contact with the apple. The limit within which the temperature can be safely changed without injuring or modifying the living organ is very small and this is one of the reasons why Bernstein's figures are not quite satisfactory, as he himself recognized; while we can change the concentration on such living objects as the apple in very wide limits without injury or complicating alterations.

We found the following method most satisfactory. An apple with perfect skin was put into a glass dish containing a small amount of a liquid *a*. On the opposite side of the apple a piece of the skin and the underlying tissue was removed and into the hole was put a small quantity of a liquid *b*. The latter remained constant throughout the experiment, while *a* was changed according to the nature of the experiment. Both liquids *a* and *b* were connected with calomel electrodes and the E.M.F. was determined by Poggendorf's compensation method (capillary electrometer).

[1] Preliminary communication.

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The temperature was kept practically constant (about 19° C.).

We, therefore, were studying the E.M.F. of the following system: liquid *a;* apple; liquid *b,* the membrane of the apple being the solid phase between liquids *a* and *b.* According to the theory a fivefold dilution of *a* should always give a constant decrease of E.M.F., namely, .040 volt; and a tenfold dilution should decrease the E.M.F. always by a value of .058 volt. These values may be expected to be smaller if the ideal conditions of semi-permeability are not fulfilled.

In the following experiment liquid *b* (in the apple) was *m*/10 KCl. Liquid *a,* on external surface, varied in concentration. The calomel electrodes contained *m*/10 KCl. The

TABLE I

Concentration of Liquid *a.*	E.M.F.
m/10 KCl	+ .040 volt
m/50 KCl	+ .068 volt
m/250 KCl	+ .103 volt
m/1,250 KCl	+ .137 volt
m/6,250 KCl	+ .169 volt

sign + means that liquid *a* was positive to liquid *b.* In this experiment each successive liquid was five times as diluted as the previous one and the theory demands that the difference of E.M.F. between two successive solutions should be identical. If we compare the interval from *m*/50 to *m*/6,250 this is true. A dilution from *m*/50 to *m*/250 increases the E.M.F. by .035 volt; from *m*/250 to *m*/1,250 by .034 volt; from *m*/1,250 to *m*/6,250 by 0.32 volt. By diluting from *m*/10 to *m*/50 we find .028 volt, which is a little too small. We have found it to be generally true that as soon as we work with more concentrated solution than *m*/50 the differences are a little smaller than the theory demands. Whether this is due to the decrease in ionization or to an injurious effect of the solutions of higher concentration upon the skin of the apple we are not yet prepared to state.

The values observed are all a little smaller than we should expect. According to Nernst's formula the difference of potential for a dilution of five should be .040 volt, while we found a difference of .033 volt. The fact that we did not get the maximum potential difference is, perhaps, due to the fact that the skin of the apple is not completely impermeable for anions.

Experiments with other salts gave similar results as far as the effect of concentration was concerned.

3. The sign of the E.M.F. of the system electrolyte; apple; electrolyte was always in that sense as if the membrane of the apple were more permeable for kations than for anions. In order to test this possibility the electromotive effects of NaCl were compared with those of Na_2SO_4. If our assumption were correct the E.M.F. of a NaCl solution should always be equal to the E.M.F. of a Na_2SO_4 of half the concentration of the former. The following example shows that this is actually the case. The internal liquid *b* remained constant throughout the experiment *m*/10 KCl. The external liquid *a* varied according to Table II.

TABLE II

Liquid *a.*	E.M.F.
m/10 NaCl	+ .038 volt
m/100 NaCl	+ .090 volt
m/1,000 NaCl	+ .139 volt
m/2,000 Na_2SO_4	+ .141 volt
m/200 Na_2SO_4	+ .092 volt
m/20 Na_2SO_4	+ .044 volt

This experiment shows that whether the anion is Cl or SO_4 as long as the concentration of the kation remains the same the E.M.F. remains unaltered. The E.M.F., if liquid *a* is *m*/1,000 NaCl, is .139 volt, and if it is *m*/2,000 Na_2SO_4 is .141 volt; practically identical values. If the liquid *a* is *m*/100 NaCl it is .090 volt, if it is *m*/200 Na_2SO_4 it is .092 volt; again practically identical values.

4. It was necessary to convince ourselves that we were not dealing with purely osmotic effects (and possibly diaphragm currents). In one experiment the external liquid *a* was *m*/100 NaCl, the liquid *b* on the injured side of the apple was *m*/10 KCl. The E.M.F. was + .092 volt. Then enough cane sugar in crystals was added to the liquid *a* to make its total concentration about *m*/2. After the sugar was dissolved the E.M.F. was .093 and

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remained so. Changes in concentration by a non-electrolyte like cane sugar, therefore, do not alter the E.M.F.

5. Continuing an investigation by Haber and Beutner on "Phasengrenzkräfte," Haber and Klemensiewicz described a concentration cell for H-ions of the type, acid; glass; alkali, the acid representing the solution with a high, the alkali with a low concentration of H-ions. Haber pointed out that this type of concentration element might correspond to the type represented by muscle, the muscle fibrils corresponding to the glass in the acid alkali element. Since the liquids in the cells are practically neutral a slight production of acid in the fibril (or the injured spot) would give rise to a considerable E.M.F. We fully expected at the beginning of these experiments to find that the E.M.F. of living organs was of the type of that found by Haber. We found, however, that for the apple this is not the case, as the following experiment shows. The internal liquid *b* was throughout the whole experiment *m*/10 KCl (neutral). The external liquid *a* was in succession *m*/20 NaCl, neutral, alkaline, acid and alkali again. It was rendered acid through addition of enough HCl to render the *m*/20 NaCl solution *m*/1,000 acid, and it was rendered alkaline through the addition of enough NaHO to render the *m*/20 NaCl solution *m*/1,000 alkaline.

TABLE III

Liquid *a*.	E.M.F.
m/20 NaCl, neutral	+ .051 volt
m/20 NaCl, *m*/1,000 alkaline	+ .052 volt
m/20 NaCl, *m*/1,000 acid ..	+ .048 volt
m/20 NaCl, *m*/1,000 alkaline	+ .052 volt

The differences found between neutral, acid and alkaline *m*/20 NaCl are slight and within the limits of purely accidental variations. If we were dealing with a reversible cell in regard to H-ions we should expect a difference of almost .5 volt between *m*/1,000 acid and *m*/1,000 alkali.

6. It may be of interest to mention also that acids and alkalis behave in regard to the E.M.F. to which they give rise like the salts. The experiments we made in this respect show also that if the concentrations of these substances are a little too high the regularity of the results suffers, and the irregularities are always of such a nature as should be expected if the injury or the etching effect of acids and alkalis increased the anion permeability of the skin of the apple. Liquid *b* in the apple was *m*/10 NaCl throughout the experiment.

TABLE IV

Liquid *a*.	E.M F.
m/10 NaCl	+ .003 volt
m/10 NaHO	+ .009 volt
m/100 NaHO	+ .041 volt
m/1,000 NaHO	+ .085 volt
m/10,000 NaHO	+ .125 volt
m/100 NaHO	+ .042 volt
m/1,000 NaHO	+ .085 volt
m/100 NaHO	+ .044 volt
m/10,000 HCl	+ .126 volt
m/1,000 HCl	+ .064 volt
m/100 HCl	+ .021 volt

The *m*/10 NaHO and the *m*/100 HCl act as if they had a slight etching effect on the skin, otherwise we notice the same influence of dilution as in the case of salts.

7. We believe that these and other experiments, which will be published in the full report, show that the influence of the concentration of electrolytes on the E.M.F. of living organs agrees quantitatively with the values to be expected if the skin is permeable for kations, impermeable or less permeable for anions.

JACQUES LOEB,
REINHARD BEUTNER
DEPARTMENT OF EXPERIMENTAL BIOLOGY,
ROCKEFELLER INSTITUTE

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The Elimination of Febrile Reactions Following Intravenous Injections of Salvarsan

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HOMER F. SWIFT, M.D.
AND
ARTHUR W. M. ELLIS, M.B.
NEW YORK

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THE ELIMINATION OF FEBRILE REACTIONS FOLLOWING INTRAVENOUS INJECTIONS OF SALVARSAN *

HOMER F. SWIFT, M.D.
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The intravenous injection of salvarsan is by far the most desirable method of administration. The reactions which follow such injections have been thought to be due to an inherent toxicity of the drug, because many of the symptoms are similar to those noted in acute arsenical poisoning. The intensity of reaction has been noted to vary to a marked extent in different places, and no doubt the severe criticism of salvarsan which has been made by various observers has been due to the marked toxic manifestations of the drug noted by them.

In our experience the reactions have varied greatly at different times. The reactions consisted usually of a chill, followed by a rise of temperature at times as high as 105 F. During the fever there was nausea and vomiting, and a severe diarrhea often followed. Intense headache and violent lumbar pains were not infrequent. Occasionally, following a high fever, a marked albuminuria with many hyaline and granular casts was noted. At one time, nearly 50 per cent. of the treatments were followed by herpes of varying intensity. In tabetics, violent lightning pains for two to six hours following the injections were almost constantly noted. They were considered evidence of a Herxheimer reaction, as the pains were usually noted in the parts of the body most affected by the tabetic process. These pains and the herpes have both been invoked as evidences of a neurotropic action of salvarsan.

* From the Hospital of the Rockefeller Institute for Medical Research, New York.

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The discovery of a method of injection whereby all of these disagreeable and dangerous symptoms are excluded is an important advance. Such a method has been found by Wechselmann.[1] This discovery followed an observation that the reactions occurring in the women's wards were less severe than those in the men's wards. Further investigation showed that the sterilization of the salt solution was carried out longer in the former wards than in the latter. He then tried the effect of distilling and sterilizing the water for the saline used in the solution of the salvarsan immediately before the treatment. After adopting this technic, no further reactions were noted. In a later communication[2] he states that he has given more than 150 injections in his office without any fever occurring within four hours after treatment. On the other hand, there was frequently a slight fall in the temperature. He considers salvarsan less toxic than mercury.

For the past six weeks we have been using freshly distilled water for the preparation of the salvarsan and normal saline, and have confirmed Wechselmann's observations. The water, with a few exceptions noted below, has been distilled within twenty-four hours of the time of the treatment. Normal saline was also prepared at the same time from freshly distilled water, and both the water and saline filtered and immediately sterilized in the autoclave. Our technic varies slightly from Wechselmann's in that we use only distilled water for the solution of the salvarsan. With saline it is often difficult to obtain a clear alkaline solution without adding a great excess of sodium hydrate. When giving the treatment, after the needle is inserted in the vein, normal saline is first injected to make certain that no leakage is occurring about the needle. The injection of salvarsan is followed by about 20 c.c. saline to wash the salvarsan from the tubing and needle before it is withdrawn.

For the past five months we have been giving repeated small intravenous injections of salvarsan as a routine. As a rule, 0.2 gm. is injected each week until a total of at least 1 gm. is administered. In some cases nearly 2 gm. have been given in this way. These small injections were frequently followed by nearly as severe reactions as doses of 0.4 or 0.5 gm.

1. Wechselmann: Deutsch. med. Wchnschr., 1911, xxxvii, 778.
2. Wechselmann: München. med. Wchnschr., 1911, lviii, 1510.

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TABLE SHOWING DIFFERENCE IN REACTIONS AFTER INTRAVENOUS INJECTIONS OF SALVARSAN

Stage	— Old Distilled Water — No Reactions	Reactions	——— Freshly Distilled and Sterilized Water ——— No Reactions	Reactions	
Primary	..	..	1	1–100.2 F.	Water and saline opened twenty-four hours previously.
Secondary	..	..	2		
Secondary	..	2	3		
Secondary	..	1	2		
Secondary	..	5	2		
Secondary	..	1	4		
Secondary	..	4	3	1–100.6 F.	Temperature of 100 F. on the previous day.
Secondary	..	2	2		
Secondary	..	2	2		
Secondary	..	2	1		
Secondary	..	2	1	2– 99.0 F. 1– 99.6 F.	Water and saline one week old.
Tertiary	..	1	1		
Tertiary	..	4	2	1– 99.0 F. 1–100.6 F.	Water fresh, saline old.
Spinal cord syphilis	1	4	3		
Spinal cord syphilis	..	4	5		
Spinal cord syphilis	..	5	4	1– 99.6 F.	Water fresh, saline old.
Spinal cord syphilis	1	4	1		
Tabes	1*	2*	3		
Tabes	..	2*	1		
Tabes	2*	1	2	1– 99.0 F. 1– 99.4 F. 1–100.6 F.	No lightning pains. No pains. Water and saline one week old. Water fresh, saline old.
Total	5	48	45	11	

* Lightning pains

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The table shows the striking difference between the old and new methods of preparation of the salvarsan.

A summary of the above shows twenty patients who received solutions prepared by both methods. A mouth temperature of 99 F. or over is considered a febrile reaction. With the former method there were forty-eight reactions in a total of fifty-three injections. With the freshly distilled water there were fifty-six injections, forty-five of which were followed by absolutely no rise of temperature or other unpleasant symptoms. Eleven treatments were followed by a temperature of 99 F. or over. Among these eleven reactions, four were only 99 F. In two with a temperature of 99.4 F. and 99.6 F., respectively, the water and saline were one week old, but both were autoclaved on the day of distillation of the water and the flasks in which they were preserved tightly capped. In three cases the water for the solution was fresh, but the saline used to precede and follow the salvarsan was some weeks old. One of these patients had a temperature of 99.6 F., and two of 100 F. Patients in another ward, who were treated with the same salvarsan solution and freshly prepared saline, had no reaction. One patient who had been previously running a slight fever had a temperature reaction of 100.2 F., and slight headache. In this instance both the water and saline had been opened twenty-four hours previously. The last reaction, 100.6 F., was in a patient who had previously been having an afternoon temperature of 100 F. In all instances with a temperature over 99 F., the reactions can be accounted for either by water or saline not absolutely fresh, or by a febrile condition of the patient. Still more striking was the absence in all the patients of nausea, vomiting, diarrhea, headache or general malaise. The patients now take their regular meals within one hour from the time of treatment. Not one of the treatments has been followed by herpes. Using the old method in patients with tabes, seven out of eight treatments were followed by lightning pains, while with the freshly prepared water in a total of nine treatments not one has been followed by any pain.

An attempt was made to find if it was absolutely necessary to have the water and saline prepared on the day of treatment. Among the forty-five treatments without reactions, two were with water and saline two days old, one seven days old and three eighteen days old.

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In all of these instances the water and saline were autoclaved on the day on which the water was distilled and the containing flasks tightly stoppered. As two slight reactions were obtained with water similarly prepared seven days previously, however, it is probably safer always to use the water as fresh as possible.

The explanation of the febrile phenomenon is not entirely satisfactory. Wechselmann[2] thinks it is due to the fact that distilled water which has stood for some time contains saprophytic microorganisms. A certain amount of proteid matter is dissolved out of these into the water. This passes through the filter and when injected into the circulation gives rise to the febrile reaction.

Whatever may be the true explanation of the febrile reaction, the fact remains that the preparation of the salvarsan with freshly distilled and sterilized water eliminates practically all unfavorable toxic symptoms. With the toxic action of salvarsan eliminated, repeated doses can be safely given and, no doubt, more effectual results will follow its more thorough application.

Sixty-Sixth Street and Avenue A.

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THE EFFECTS OF SUBDURAL INJECTIONS OF LEUCOCYTES ON THE DEVELOPMENT AND COURSE OF EXPERIMENTAL TUBERCULOUS MENINGITIS.*

BY WILFRED H. MANWARING, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

The therapeutic control of tuberculosis has still to be accomplished. Of the specific therapeutic agents thus far proposed, tuberculin alone seems to exercise a favorable influence over certain forms of the disease. The results of sanatorium and climatic treatment, however, have amply demonstrated that there are in the body natural curative powers against this disease. While the mechanism of the natural cure has not as yet been fully worked out, it would nevertheless appear that phagocytic cells play an important part in it. It therefore becomes of interest to determine the extent of the action of the body phagocytes under controllable experimental conditions. The leucocytes are the phagocytic cells most readily obtained for experimental purposes.

Kling[1] has shown that leucocytes, injected simultaneously with tubercle bacilli, have the power of decreasing the pathogenic properties of these microörganisms. To Opie,[2] however, belongs the credit of a painstaking and systematic study of the effects of injected leucocytes upon a tuberculous lesion already developed. Opie inoculated the pleural cavity of dogs with tubercle bacilli, and found that leucocytes, brought into contact with the tuberculous tissue thus produced, made very striking alterations in it. The flat plaques of tuberculous tissue upon the pleura of the control ani-

* Received for publication, November 1, 1911.

[1] Kling, Untersuchungen über die bakterientötenden Eigenschaften der weissen Blutkörperchen, *Ztschr. f. Immunitätsforsch.*, 1910, vii, 1.

[2] Opie, The Effect of Injected Leucocytes upon the Development of a Tuberculous Lesion, *Jour. Exper. Med.*, 1908, x, 419.

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mals, were almost absent in the treated dogs. The pleura was sometimes returned even to the delicate translucent appearance observed in the normal animal. The masses of tuberculous tissue in the mediastinum and in the sub-pericardial membranes were also smaller after the injections, and there were fewer metastatic lesions in the extrathoracic organs.

The work embodied in this paper is part of the study of the experimental therapeutics of meningeal infections,[3] which is being carried out at the Rockefeller Institute. The cerebrospinal membranes differ from the true serous membranes in that they are almost impervious to the blood proteids, and normally receive little or no protective substance from this source, and few, if any, leucocytes. The meningeal cavity is, therefore, always in an inferior condition of defense against infection. The method of lumbar puncture, however, provides a ready means of bringing healing substances into direct contact with pathological processes existing within these membranes, and has made possible a study of the local therapeutics of diseases of this surface. The experiments were made on dogs and consisted, first, in producing a tuberculous infection of the meninges, and, second, in attempting to control the lesion by the local injection of dog leucocytes.

METHOD.

Inoculation of the subdural cavity in the dog is attended with some difficulty. The spinal cord of this animal extends throughout the entire spinal canal, making the introduction of a needle, as for lumbar puncture, impracticable. Injury of the cord follows such introduction, and the resulting paralyses and other secondary effects rule out this method of procedure. A trephine opening in the cranium was therefore substituted.[4]

In devising the method, it was borne in mind, not only that inoculation of tubercle bacilli was to be provided for by this means, but also that provision must be made for the subsequent injection of

[3] Flexner, The Biological Basis of Specific Therapy, *Boston Med. and Surg. Jour.*, 1911, clxv, 709.

[4] All the operative procedures were carried out under morphin-ether anesthesia.

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leucocytes. This was achieved by dividing the operation into two stages. In the first stage, the trephine opening was made, the button of bone removed was replaced by a disk of paraffin, the wound closed by cat-gut sutures, and allowed to heal under an antiseptic dressing. As soon as healing was complete, the actual inoculation was made, by passing a long, slender, blunt hypodermic needle through the skin, muscle, and paraffin disk, into and through the frontal lobe of the brain to the base of the skull. Leakage into the subcutaneous tissue was prevented by pressure on the paraffin on withdrawing the needle.

Preliminary experiments with suspensions of Berlin blue showed that a fluid so injected distributes itself quite uniformly over the base of the skull, extending a quarter, even a third of the way down the spinal meninges.

Several strains of tubercle bacilli were thus inoculated:

(1) Bovine. Originally obtained from Dr. M. P. Ravenel. Extremely virulent.

(2) Human (H39). Obtained from Dr. E. L. Trudeau. Virulent.

(3) Human (H24). Obtained from Dr. Theobald Smith. Slightly less virulent.

(4) Human (H38). Stock culture, Rockefeller Institute. Avirulent for dogs. Subdural injections of this culture at no time caused symptoms of meningitis. The bacilli gradually decreased in number and, while still present in considerable numbers as late as the fortieth day after injection, had disappeared by the sixtieth day.

The inoculation material was prepared by filtering emulsions of these cultures through closely packed absorbent cotton, and diluting the resulting filtrates to the desired turbidity. Bacterial emulsions so filtered are practically free from clumps. As a turbidity standard, a $\frac{1}{20}$ per cent. lecithin suspension was used, prepared by diluting a 1 per cent. alcoholic solution of lecithin with nineteen volumes of physiological saline. The volume of fluid usually injected was two cubic centimeters of the bacterial suspension, followed by one cubic centimeter of physiological saline to wash out the needle, a separate syringe barrel being used for the salt solution.

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EXPERIMENTAL TUBERCULOUS MENINGITIS.

The injection of virulent tubercle bacilli into the subdural space of dogs by the above technique, produces a disease characterized by a latent or incubation period of from five to thirty days, followed by a period of increasing paralysis and incoördination that terminates, almost invariably, in death. The clinical picture, during the paralytic stage, varies in different animals, depending on the groups of muscles most involved, and on whether the paralyses are of the flaccid or of the spastic type. In order to record these paralyses, the following symbols have been adopted:

 = Apparently normal; no paralysis, no incoördination.

 = Beginning paralysis, beginning incoördination. Such an animal may show a slight spastic condition of the posterior extremities, or a tendency to stumble on running. A slight arching of the back and a beginning tenseness of all the trunk muscles are characteristic of this period.

 = Well-marked paralyses and incoördinations. Such an animal may have all its extremities either in a flaccid or a spastic condition. Its movements may be so incoördinate that the animal falls to its side; the neck and back may be held rigidly and the animal may cry out on being handled.

 = Extreme paralysis, collapse. The animal in this stage is unable to stand. It usually lies on its side, refuses food, and rapidly emaciates. The back and neck are usually held rigidly.

 = Death.

The following symbols have been adopted to represent symptoms unassociated with paralyses and incoördinations:

 = Quieter than normally. The animal refuses to play, spends most of its time curled up in a corner of the cage, and may object slightly to being handled.

 = Very quiet, weak, objects to being handled.

 = Profound stupor, collapse.  = Death.

A typical course of the disease as represented by these symbols is shown in figure 1.

For the purpose of the present paper, the slight daily variations in the paralysis are of little moment. To obtain a less detailed

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FIG. 1. TYPICAL COURSE OF TUBERCULOUS MENINGITIS. A fox-terrier, weighing 8 kilos, trephined 16 days previously. The wound healed by first intention. 0.1 c.c. of a suspension of bovine tubercle bacilli in 2 c.c. of physiological saline was injected, followed by 1 c.c. of salt solution to wash out the needle. The animal showed an incubation period of 11 days, mild constitutional symptoms beginning on the 12th day, definite paralysis on the 15th day, increasing to collapse on the 25th day; death on the 28th day. Autopsy showed a tuberculous process involving the entire meningeal cavity; abdominal and thoracic viscera negative.

picture, the above symbols have been combined,



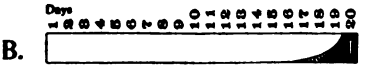
and the daily variation eliminated by changing the profile to a smooth curve.



This method of representation has been adopted in the subsequent parts of the paper.

The above case, selected as the typical course of the disease, is characteristic of the majority of cases. About a quarter of the animals, however, do not conform to this type. For the sake of presenting these atypical cases, they are here divided into four groups. In these groups are included only uncomplicated cases of tuberculous meningitis, animals developing distemper and other intercurrent infections being carefully excluded from these records.

The first of these atypical cases is characterized by a slightly prolonged latent period, followed by sudden death, with or without slight preliminary paralytic symptoms. The animals are usually quieter than normally for a day or two before death. Death is usually preceded by convulsions and coma.



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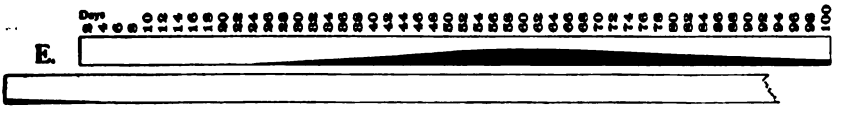
The second atypical group is characterized by an unusually prolonged paralytic stage. Animals of this type usually show the flaccid, rather than the spastic type of paralysis. The head and neck muscles are usually but little involved, thus interfering only slightly with the movements necessary for taking food.



The third group is characterized by a remission or actual disappearance of the paralytic symptoms, followed by their reappearance and death.



In the fourth group are included the spontaneous recoveries from the disease.



On account of the existence of these atypical cases, care must be exercised in drawing conclusions from clinical data, since parallel animals, inoculated with equal doses, may give quite different clinical pictures (figure 2).

To reduce these variations to a minimum, care was exercised in



FIG. 2. CLINICAL VARIATIONS. Two fox-terriers inoculated with equal doses (0.3 c.c.) of bovine tubercle bacilli. In the first animal (F), definite paralysis began on the 6th day, progressed to collapse on the 11th day; death on the 15th day. The second animal (G) showed no paralytic symptoms till the 20th day. Collapse on the 21st day; death on the 22d.

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choosing animals for parallel inoculations. The animals selected were as nearly as possible of the same weight, the same breed, the same age, and in the same condition of nutrition. For most of the experiments, large, well nourished fox-terriers and bull-terriers were selected. Collies, spaniels, Irish terriers, and other long-haired dogs were avoided, as they were found irregular in their reactions and very susceptible to intercurrent infections. Young dogs were also avoided for the same reason. By exercising this care, it was found possible to produce approximately the same clinical picture in parallel inoculations (figure 3).

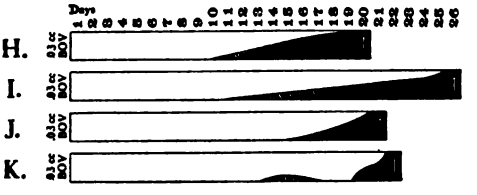


FIG. 3. PARALLEL INOCULATIONS. Four carefully selected adult fox-terriers, inoculated with equal doses of tubercle bacilli, showing comparatively slight variations in the clinical picture.

The clinical manifestations varied with the virulence of the culture injected (figure 4). With the very virulent bovine culture, the incubation period was short, the development of the paralytic symptoms rapid, and the outcome invariably fatal. With the less virulent human cultures, the incubation period was prolonged, the paralytic symptoms developed slowly, and an occasional animal recovered spontaneously.

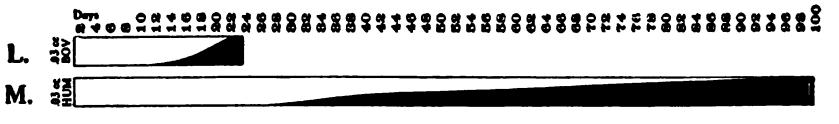


FIG. 4. RELATION TO VIRULENCE. Parallel inoculations with equal doses of bovine (L) and human (M) tubercle bacilli.

The clinical manifestations also varied with the dosage (figure 5). The latent period was perceptibly prolonged on decreasing

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the dose, while with the smaller doses the paralytic symptoms developed slowly and there was a greater number of spontaneous recoveries.

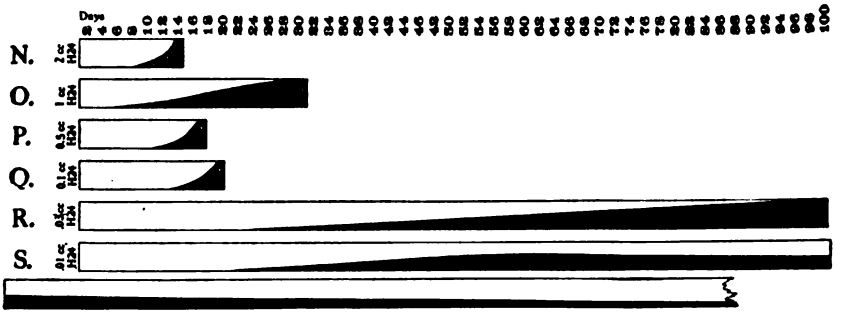


FIG. 5. RELATION TO DOSAGE. Parallel inoculations with decreasing doses of the same organism.

The local anatomical changes in the disease varied with the virulence and dosage of the organism injected. There was always more or less thickening of the meninges, with adhesions to adjacent structures. The subdural space usually showed an active tuberculous process, varying from an accumulation of fluid, fibrin, and semi-necrotic cells in the more acute cases, to well organized masses of diffuse tuberculous tissue in the more chronic cases. Smears made from the subdural space showed tubercle bacilli. There was usually a considerable accumulation of round cells about the cerebral and spinal capillaries, with occasional areas of necrosis.

The other organs of the body usually showed no characteristic change. Miliary tubercles were found in the livers of about a quarter of the dogs injected with the bovine culture, more rarely in the lungs and other organs. Such nodules, however, were never found in dogs injected with the human strains.

This does not necessarily mean that the bacilli of the human type did not occasionally escape from the meningeal cavity into the general circulation, but it indicates presumably only that the virulence of the human cultures was too small for the few that did escape to set up the disease in the extrameningeal tissues. This view is strengthened by experiments, in which small doses of the bovine

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and human cultures were injected intravenously. With the bovine culture, a generalized miliary tuberculosis was always produced by this means. With the human cultures, such nodules were never formed.

Dogs recovering spontaneously from the paralytic symptoms showed, a few months later, simply a slight opacity of the meninges, with a few old adhesions. No tubercle bacilli were then found.

EFFECTS OF LEUCOCYTIC INJECTIONS.

The leucocytes used in treating the infected animals were obtained by the method perfected by Opie.[5] Five days before the proposed treatments, one cubic centimeter of turpentine was injected into the pleural cavity of a normal dog, and three days later a second injection of a similar amount was given. The resulting rapidly formed serous effusion was withdrawn and discarded on the fourth day, following which withdrawal a pleural exudate very rich in leucocytes was usually formed. This was used on the fifth day.

To prevent clumping, this exudate was drawn into about a third of its expected volume of 1.5 per cent. sodium citrate. The exudate was then filtered through gauze, freed from plasma by centrifugation, washed with salt solution, refiltered if necessary to remove lumps, and made up into a 50 per cent. suspension in physiological saline.

A microscopic examination of suspensions so prepared showed them to consist of mononuclear and polymorphonuclear leucocytes, mixed with a few red cells. Bacteria were never seen. Routine sterility tests, by transferring a sample to nutrient agar, gave uniformly no growth. About a third of the leucocytes stained immediately on the addition of very dilute methylene blue, from which it was concluded that these cells were probably necrotic, although still preserving their normal microscopic appearance.

In making the subdural leucocytic injections, the same technique was employed as in the initial subdural inoculations with tubercle bacilli; a long, slender, blunt hypodermic needle was passed through

[5] Opie, *loc. cit.*

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the paraffin disk filling the permanent trephine opening, into and through the frontal lobe of the brain, and the leucocytes were forced into the basal meninges. The volume of suspension thus injected was usually about four cubic centimeters for fox-terriers, and six cubic centimeters for bull-terriers. Occasionally as much as ten cubic centimeters have been injected with apparently no ill effects.

The usual immediate effect of the injection was a temporary cessation of respiratory movements. As a routine, the animal was at once placed under artificial respiration, the tongue and epiglottis being retracted to insure a free respiratory passage. Spontaneous respiratory movements were usually resumed within two minutes, but were occasionally delayed for five or ten minutes, or even longer.

The later effects of the injections were less satisfactory. About a quarter of the animals died within a few hours. In a number of cases, death was attributable to edema of the lungs, or to unsuspected pneumonia from earlier etherizations; but many of the autopsies showed apparently no such accessory cause.

For several days after the injection, a number of the surviving animals were quiet, showed evidences of cranial tenderness, a few even showing disturbances in equilibrium, circulatory movements, and the like. These symptoms usually disappeared within two or three days, but occasionally persisted for a longer period. The majority of the animals, however, showed no symptoms the day after the injection.

The effect of the leucocytic injections varied with the dosage and virulence of the organism used. With large doses of tubercle bacilli the leucocytes were apparently without effect (figure 6).



FIG. 6. EFFECT OF LEUCOCYTIC INJECTIONS. Composite picture of four treated dogs (W) and four untreated controls (w). In making these composites, the average day on which symptoms of a certain severity first made their appearance was taken as the day of its appearance in the composite animal. The number of cubic centimeters of leucocytic suspension injected is indicated by the small exponents; thus, $L^6 = 6$ c.c.

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With small doses of the very virulent bacilli of the bovine type (figure 7), the injections produced a uniform prolongation of the latent period of the disease, but had a less evident influence upon the subsequent course of the paralytic symptoms. Possibly the paralytic symptoms were somewhat less acute in the treated dogs than in the untreated controls, but the number of experiments was too small to establish this beyond question. All animals of this series died as a result of the meningeal infection.

With small doses of the less virulent human cultures, the injections had an even greater influence (figure 8). The control animals, inoculated with these doses, invariably developed paralytic and ataxic symptoms, usually after an incubation period of about four weeks. The resulting paralyses usually increased slowly in severity, and in half the cases terminated fatally in about three months. In the other half, the paralyses were of a more chronic type, persisting to the present date (seven months). Half of the animals receiving the leucocytic injections have shown no paralytic symptoms up to the present day (seven months), while the others have developed paralyses only after very prolonged incubation periods, from which one animal of the series has thus far died.

The local histological changes produced by the leucocytic injections can be determined only by killing animals at various stages after such injections. This has not been done. What can be stated, however, is that when fatal paralyses develop in the treated animals, the autopsy usually shows approximately the same histological changes as in the untreated controls. In some of the treated dogs, however, the tuberculous lesion in the upper parts of the subdural space has apparently been arrested, or caused to undergo resolution by the leucocytic injections, while persisting or advancing to a chronic condition about the lower third of the spinal cord, to which situation the leucocytic suspension presumably did not penetrate.

The study of the therapeutic control of tuberculous meningitis is made difficult in the dog by the necessity of making the injection and treatment through a trephine opening. Danger exists of producing fatal pressure effects, and the frequent etherizations expose the animal to intercurrent respiratory infections. Moreover, the

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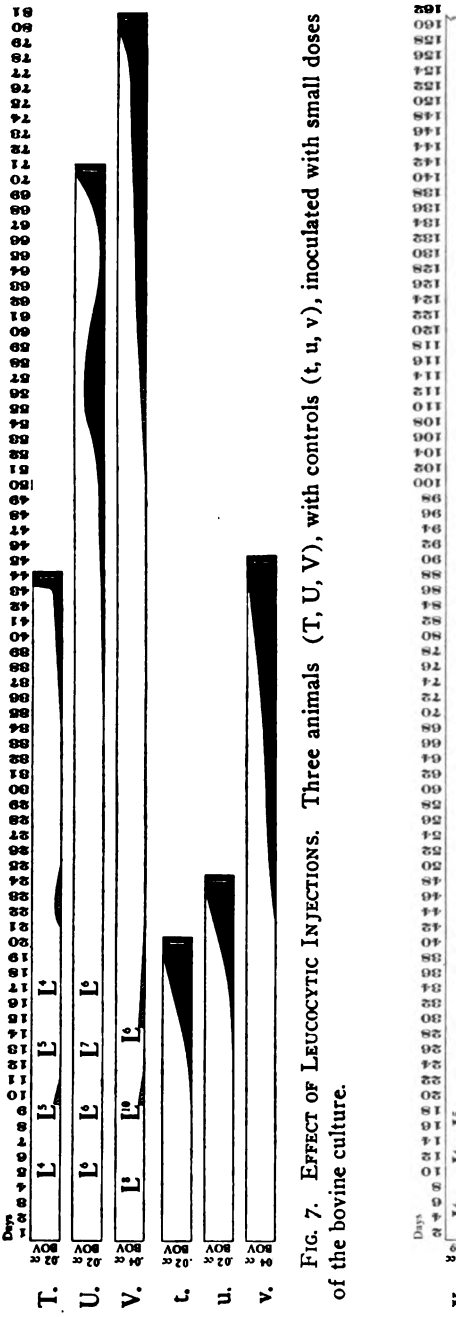


FIG. 7. EFFECT OF LEUCOCYTIC INJECTIONS. Three animals (T, U, V), with controls (t, u, v), inoculated with small doses of the bovine culture.

FIG. 8. EFFECT OF LEUCOCYTIC INJECTIONS. Comparison as in figure 7, but with animals injected with small doses of the less virulent bacilli of the human type.

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medicament introduced into the cranial cavity penetrates with difficulty to the caudal levels of the spinal cord, leaving that part of the cord practically untreated. It is intended therefore to pursue this investigation upon animals in which the inoculations and injections can be made with less traumatic injury and under conditions more nearly approaching those occurring in spontaneous tuberculous meningitis in human beings.

The later history of the animals now under observation will be reported in a subsequent paper.

SUMMARY.

Subdural inoculation of tubercle bacilli of established virulence for guinea pigs and rabbits, produces in the dog a tuberculous meningitis, followed by paralysis and death. When suspensions of canine leucocytes are injected subdurally, following such inoculations there occurs uniformly a delay in the development of the paralysis and a prolongation of the life of the treated animal. In dogs inoculated with small doses of tubercle bacilli of low virulence, the development of paralysis has been prevented by this means for periods of seven months (up to the present date), while the untreated animals injected with the same cultures have all developed paralytic symptoms within a period of about four weeks.

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STUDIES WITH THE ELECTROCARDIOGRAPH ON THE ACTION OF THE VAGUS NERVE ON THE HUMAN HEART

By G. CANBY ROBINSON, M.D., and GEORGE DRAPER, M.D.

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STUDIES WITH THE ELECTROCARDIOGRAPH ON THE ACTION OF THE VAGUS NERVE ON THE HUMAN HEART.

II. THE EFFECTS OF VAGUS STIMULATION ON THE HEARTS OF CHILDREN WITH CHRONIC VALVULAR DISEASE.*

BY G. CANBY ROBINSON, M.D., AND GEORGE DRAPER, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

PLATES 1–5.

During the study that forms the subject of the present communication, the methods and conditions were constantly similar to those described in our first paper (1). The electrocardiograms were always obtained by electrodes attached to the right forearm and left leg—the so-called second lead. The string of the galvanometer was adjusted so that its shadow moved one centimeter on the scale attached to the camera when a current of one millivolt passed through it.

The hearts of children suffering with so-called chronic endocarditis were found to be very susceptible to vagus stimulation by pressure. The study was carried out on a group of four children, in all of whom acute articular rheumatism can probably be held accountable for the heart lesions.

Case I. A girl of eight years, who first gave evidence of heart disease when six years old at the time of an attack of purpura. The diagnosis at the time of admission was mitral insufficiency, probably also aortic insufficiency and chronic pericarditis. The pulse rate ranged from 110 to 150 per minute while quiet in bed, and the blood pressure was estimated by the auscultatory method at systolic 105, diastolic 25 millimeters of mercury.

Case II. A boy of fourteen years, whose cardiac symptoms developed at the age of nine, two years after acute articular rheumatism. He suffered from mitral and aortic insufficiency. The pulse rate was from 100 to 126 per minute, and the blood pressure was systolic 110, diastolic 54 millimeters of mercury on admission, and on the next day the systolic pressure was 130.

* Received for publication, October 11, 1911.

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Case III. A girl of seven years, who first showed cardiac symptoms during her fifth year after tonsillitis and diphtheria. Later she had acute articular rheumatism and presented the signs of mitral insufficiency. Her heart rate was 110 per minute on admission.

Case IV. A girl of fifteen years, who, besides having had rheumatism, gave a positive Wassermann reaction and whose parents gave a definite history of syphilis. Her cardiac symptoms appeared during her ninth year and the diagnosis of mitral insufficiency and congenital syphilis was made. Her pulse rate was 75 to 85 while quiet in bed, and her blood pressure was systolic 98, diastolic 60 millimeters of mercury.

In each case, the heart was beating with sufficient force to give a very well defined apex impulse, and in the first three cases, in which the rate was abnormally rapid, the heart action may be described as thumping. All the cases showed a marked enlargement of cardiac dullness on percussion. It is, of course, impossible to say what myocardial changes were present, but it is reasonable to suppose that the heart muscle, especially in the case where both rheumatism and congenital syphilis had been present, was at least physiologically abnormal. All the curves to be presented were obtained when the patients were free from the effects of digitalis.

The curves will first be described and the various features of the entire group will then be discussed.

The electrocardiograms have been analyzed in order to determine how the vagus stimulation has affected the various cardiac properties—rhythmicity, conductivity, contractility, and excitability—and the results of the analyses have been tabulated as previously. The measurements are given in seconds, calculated from the markings of the Jacquet chronograph, except those of the heights of waves which are in millimeters. The duration of each ventricular cycle is determined by measuring the time elapsing between the first evidence of ventricular stimulation in the electrocardiograms. This time is designated Q–Q or R–R in the tables, depending on whether the initial ventricular stimulation produced a negative Q wave or a positive R wave. The conduction time is measured by computing the time elapsing from the onset of auricular stimulation, the foot point of the P wave, to the onset of ventricular stimulation. This time is therefore represented by P–Q or P–R in the tables. In the same way, the length of auricular cycles is designated as P–P. Ventricular systole is measured from the onset of the Q or R wave to the end of the T wave, while from this last point to the following Q or R point represents ventricular diastole.

Figures 1 to 6 are from case I. Figure 1 shows one cycle of the electrocardiogram obtained when the heart was beating at its usual rapid rate, 140 per minute, undisturbed by vagus stimulation. The other eight cycles in this curve are very similar, and the measurements average as follows:

$$Q\text{–}Q = 0.430, \quad P\text{–}Q = 0.152, \quad P = 3.6, \quad R = 18.2, \quad T = 3.2.$$

No comment is needed except to call attention to the rapidity of rate, which causes a partial fusion of the T and P waves, and the prominence of these waves.

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Figure 2 shows the effect of pressure on the right vagus nerve, figure 3 on the left vagus. The measurements from these curves are given in the following tables.

FROM FIGURE 2.

Curve 27.1. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	Systole.	Diastole.	*P–Q.*	*P.*	*R.*	*T.*
1	——	——	——	0.152	2.5	15.7	2.2
2	0.458	——	——	0.157	3.0	16.5	2.2
*3	0.422	——	——	0.162	2.7	14.5	2.0
4	0.400	0.282	0.218	0.162	2.7	15.5	2.0
5	0.972	0.282	0.690	0.151	2.2	15.0	2.5
6	0.997	0.323	0.674	0.161	1.5	15.0	2.5
7	0.782	0.298	0.484	0.150	1.0	15.3	2.5
8	0.653	0.304	0.349	0.164	1.2	16.0	2.5
9	0.590	0.297	0.293	0.150	2.0	15.5	3.0
10	0.575	0.297	0.278	0.165	2.0	—	3.0
11	0.537	0.290	0.247	0.157	2.2	16.0	3.0
12	0.532	0.292	0.240	0.161	2.5	15.5	2.7
13	0.500	0.294	0.206	0.158	2.5	15.5	2.5
14	0.495	0.297	0.198	0.149	2.5	16.0	2.3

* Vagus pressure was made at the point of the first asterisk and was released at the point of the second asterisk in all curves where the release is indicated.

FROM FIGURE 3.

Curve 27.2. Left Vagus Pressure.

Number of cycle.	*Q–Q.*	Systole.	Diastole.	*P–Q.*	*P.*	*R.*	*T.*
1	——	——	——	0.149	3.0	18.5	3.0
2	0.462	——	——	0.153	3.2	18.0	—
*3	0.446	——	——	0.160	3.5	17.5	3.0
4	0.454	——	——	0.160	3.5	17.2	3.0
5	0.478	——	——	0.168	3.3	18.5	3.0
6	0.497	——	——	0.186	3.5	18.2	3.0
7	0.546	0.301	0.245	0.249	3.5	19.0	3.0
8	0.629	0.288	0.341	0.227	3.3	18.5	3.0
9	0.710	0.298	0.412	0.249	3.5	21.5	2.5
10	0.654	0.309	0.345	0.264	3.5	21.5	3.0
11	0.626	0.314	0.312	0.228	3.5	19.5	2.5
12	0.643	0.299	0.344	0.246	3.0	—	3.0
*13	0.600	0.300	0.300	0.217	3.5	20.0	3.0
14	0.535	0.310	0.225	0.183	3.5	18.5	3.0(?)
15	0.541	0.310	0.231	0.169	3.5	19.5	3.5
16	0.526	0.304	0.222	0.165	3.0	20.0	3.5
17	0.503	0.309	0.194	0.162	3.0	20.5	3.0
18	0.495	0.290	0.205	0.150	3.0	19.0	3.0
19	0.475	0.299	0.176	0.145	3.5	19.0	3.0
20	0.490	——	——	——	3.5	—	—

On another occasion, pressure was made first on the right vagus and then on the left with practically similar results, as seen by the following measurements of the curves which are not reproduced.

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CURVE NOT REPRODUCED.

Curve 24.2. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	——	——	——	17.8	3.2
2	0.432	0.142	2.5	16.5	3.5
3	0.433	0.148	2.5	16.5	2.8
4	0.433	0.147	2.5	——	3.8
*5	0.439	0.157	3.0	17.5	3.2
6	0.479	0.155	3.0	17.3	3.4
7	1.768	0.217	1.7	19.0	3.5
8	0.657	0.211	1.8	18.5	4.5
9	0.653	0.175	1.8	18.0	4.0
10	0.711	0.192	2.0	19.0	4.5
11	0.719	0.164	2.0	18.8	4.3
12	0.635	0.167	1.8	18.2	5.0
13	0.592	0.133	2.0	18.2	5.0
*14	0.597	0.158	2.0	18.5	4.5
15	0.530	0.135	2.5	18.5	5.0
16	0.511	0.155	2.5	18.2	4.5
17	0.492	0.154	2.5	18.6	4.5
18	0.488	0.158	3.0	18.0	4.2
19	0.467	0.158	2.7	17.6	—

CURVE NOT REPRODUCED.

Curve 24.3. Left Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	——	0.152	3.0	18.0	3.5
2	0.433	0.148	2.7	18.0	4.0
3	0.438	0.152	3.0	18.5	3.5
*4	0.436	0.154	3.0	17.0	3.5
5	0.431	0.151	3.0	18.5	3.5
6	0.438	0.153	3.0	18.5	4.0
7	0.453	0.167	3.0	18.5	3.5
8	0.524	0.223	3.5	19.0	3.0
9	0.522	0.219	3.0	19.0	3.0
10	0.542	0.208	3.0	19.0	3.5
11	0.472	0.190	3.5	19.0	4.0
12	0.462	0.177	3.0	19.0	4.0
13	0.464	0.184	3.5	18.0	4.0
14	0.463	0.169	3.0	18.0	4.0
15	0.482	0.166	3.0	18.5	——
*16	0.459	0.160	4.0	18.5	4.0
17	0.474	0.173	3.5	18.0	4.0
18	0.464	0.169	3.0	18.0	4.0
19	0.462	0.163	3.5	18.0	4.0

It is seen from the measurements in both sets of curves that there has been a marked difference between the effect of stimulation of the right and left vagus. Right vagus pressure had a striking effect on the heart rate, apparently through its action on the sinus node, the normal "pace-maker" of the heart-beat. The slowing came on less than a second after pressure was made and caused the maximal lengthening of the cardiac cycle in the first or second slow

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beat. Left vagus pressure caused but slight slowing, and the longest cycle in each curve was the seventh after the pressure was exerted, each occurring about three seconds after the initial pressure. The time of conduction of the heart-beat from auricles to ventricles, as indicated by the *P–Q* time, is, however, more influenced, both relatively to the heart rate, and absolutely, by stimulation of the left vagus than the right. In the curve 27.1 (figure 2) right vagus pressure produced no delay in conduction, although the slowing of the cardiac rhythm was marked. Pressure on the left vagus nerve caused marked delay in conduction on both occasions, in curve 27.2 (figure 3) with moderate, and in the unpublished curve, 24.3, with almost no change in rate. The changes in conduction occurred synchronously in the curves with the changes in rate. Right vagus stimulation caused a striking diminution in the size of the *P* wave, which was apparently unaffected by left vagus pressure. The *R* wave was usually increased, slightly more by left-sided pressure, and the *T* wave more by right-sided, but not until several beats after pressure was made.

On two occasions in case I, right vagus pressure produced a dissociation of auricles and ventricles, seen in figure 4. The measurements from this curve are as follows:

FROM FIGURE 4.

Curve 19.2. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P–P.*	*P.*	*R.*	*T.*
1	0.421	——	——	——	——	3.5
2	0.424	0.149	0.424	4.0	19.5	3.5
3	0.434	0.149	0.435	4.0	18.0	3.5
4	0.420	0.148	0.419	4.0	18.0	3.3
5	0.420	0.149	0.420	4.0	19.0	3.0
6	0.433	0.149	0.431	3.8	19.5	3.3
*7	0.670	0.151	0.658	3.8	18.8	3.7
8	0.687	0.170	0.661	2.5	20.8	3.5
9	0.991	0.196	1.011	3.0	21.2	3.8
10	°0.853	0.176	0.991	1.8	20.0	4.4
11	°0.788	——	1.000	——	20.0	5.0
12	°0.794	——	0.700	——	22.8	4.0
13	°0.778	——	0.706	——	21.8	4.5
14	°0.780	——	0.674	——	24.0	5.0
15	0.635	0.137	0.596	——	21.3	5.2
16	0.556	0.133	0.640	3.3	21.5	5.0
17	0.517	0.141	0.509	3.3	20.0	5.0
18	0.489	——	0.493	3.0	20.0	4.3

° = independent ventricular cycles. Independent ventricular rate = 75.1.

It is seen that after definite slowing in rate, but without maximal delay (as compared to figure 3), the *R* and *P* waves occur in abnormal relations in cycles 11, 12, 13, 14, and 15. The relations of the waves then resume that seen before vagus pressure was exerted, except for a shortening in the conduction time. It seems evident that this temporary disturbance in the electrocardiogram results from a complete dissociation of auricles and ventricles. The ventricles take up their own pace at a rate of 75.1 beats per minute, but the first independent ven-

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tricular cycle is longer than those that follow. Practically the same ventricular complex is seen during as before and after the dissociation. The changes in the *R* waves in this part of the curve seem referable to the coincidences of the *P* and *R* waves. Otherwise the changes in the various waves are similar to those previously seen, except that there is a well defined increase in the *T* wave.

Figures 5 and 6 are reproduced to show the effect that right vagus pressure had on the cardiogram, taken by means of an open funnel held over the apex beat. The child had a thin chest wall, and the violently beating heart produced a very well defined apex beat. In figure 5, the cardiogram is recorded synchronously with the electrocardiogram. Soon after the initial pressure over the right vagus, which took place between the fifth and sixth cardiac cycles, the cardiogram becomes diminished in height until the thirteenth cycle is reached, when it is seen to have almost disappeared. The appearance of the entire length of this curve and of a number of others that were taken, make it certain that respiratory movements are in no way responsible for this flattening out of the cardiogram. Greatly weakened ventricular contractions seem to be the only possible cause. The marked diminution in intensity of the cardiac sounds and murmurs, which invariably occurred, confirm this explanation. The late appearance of the maximal weakening of the ventricles is worthy of note. It occurs over four seconds after the initial pressure and does not accompany the maximal slowing or depression of conductivity. The measurements from the curve show, however, neither a marked decrease in rate nor much delay in conduction.

FROM FIGURE 5.

Curve 19.5, with Apex Beat. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.436	0.149	—	——	—
2	0.430	0.154	3.5	18.0	3.5
3	0.437	0.152	3.5	18.0	3.5
4	0.432	0.150	3.0	18.0	3.5
5	0.442	0.154	3.5	18.0	—
*6	0.470	0.161	—	18.3	4.0
7	0.550	0.171	4.0	17.5	3.5
8	0.582	0.174	3.0	18.0	—
9	0.652	0.169	2.7	20.0	4.0
10	0.652	0.165	2.8	20.0	4.0
11	0.731	0.155	2.8	19.5	4.3
12	0.670	0.157	2.4	19.5	4.5
†13	0.637	0.160	2.5	21.0	—
14	0.635	0.156	2.0	20.5	5.5 (?)
15	0.560	0.155	3.0	19.0	4.5
16	0.556	0.168	2.5	20.0	5.0
17	0.536	0.155	3.0	19.5	4.5
18	——	——	2.7	19.0	4.0

† Greatest decrease in size of apex beat.

It is also interesting to find that the *R* wave of the greatest height occurs with the most weakened beat, while the greatest depression of the *P* wave is seen in the following beat. The definite increase in the *T* wave is seen in the latter part of the curve, as in the others, when the right vagus was pressed upon.

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Figure 6 shows tracings from the apex beat and brachial artery, recorded in the usual way on the smoked paper drum of a kymograph. Right vagus pressure made while the curve was being taken, produced the same diminution in the cardiogram. The slowing of the heart responds to right vagus stimulation in what seems to be the usual way, while it is the second beat after the longest pause that forms the smallest cardiogram. The explanation for the fact that the weakest beat and the longest pause do not occur synchronously here may lie in the fact that the long rest afforded the heart had caused a stronger ventricular beat than occurred when the rest was not so long. This reasoning, however, can hardly be applied to explain the non-parallelism in rhythmicity and contractility which is seen in figure 5. Only a part of a long graphic record is reproduced, but when the entire curve was studied, respiratory effects could be excluded as the cause of the depression of the cardiogram. Cold and then hot applications to the neck of this patient, for about an hour and a quarter each, failed to alter the heart rate or the susceptibility of the heart to vagus pressure.

Figures 7, 8, 9, and 10 are from case II. In the record obtained without vagus pressure (figure 7), it is seen that the *P*, *R*, and *T* waves are very well marked, the *T* wave being abnormally large. Measurements of the entire curve of eight cycles, of which only part is reproduced, average as follows:

$$Q\text{–}Q = 0.553,\quad P\text{–}Q = 0.174,\quad P = 2.3,\quad R = 21.6,\quad T = 4.5.$$

The rate was 109 beats per minute. A slight depression of conductivity is indicated by the *P*–*Q* time of 0.174.

The difference in the effects of stimulation of the right and left vagi is seen by comparing figures 8 and 9.

FROM FIGURE 8.

Curve 41.2. Right Vagus Pressure.

Number of cycle.	*Q*–*Q*.	*P*–*P*.	*P*–*Q*.	*P*.	*R*.	*T*.
1	0.511	0.503	0.172	2.5	21.5	4.0
2	0.512	0.523	0.184	2.5	21.5	5.0
*3	0.572	0.584	0.173	2.5	22.0	4.5
4	0.834	0.811	0.161	2.5	22.0	4.5
5	0.777	0.799	0.184	1.0	22.5	4.0
6	0.789	0.792	0.162	1.0	22.0	4.2
7	0.823	0.815	0.159	1.0	22.0	4.5
8	0.800	0.805	0.167	—	22.5	4.5
*9	0.796	0.794	0.162	0.5	22.5	5.0
10	0.601	0.608	0.164	1.0 (?)	21.5	5.0
11	0.595	0.582	0.157	1.0	22.0	5.0
12	0.561	0.564	0.170	2.0	21.5	4.5
13	0.543	0.546	0.167	2.0	22.5	5.0
14	0.515	0.498	0.164	2.0	22.5	5.0
15	0.497	0.505	0.181	2.0	23.0	4.7
16	0.508	0.508	0.173	2.0	23.0	4.5
17	0.535	0.600	0.173	2.5	23.0	4.5
18	—	—	0.157	—	—	—

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FROM FIGURE 9.

Curve 41.6. Left Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–P.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.524	0.520	0.171	2.5	23.0	4.5
2	0.524	0.522	0.175	2.5	22.3	4.5
*3	0.560	0.576	0.177	2.5	21.0	4.5
4	0.512	0.502	0.161	2.3	22.5	4.5
5	0.706	0.714	0.171	2.5	21.5	4.5
6	0.530	0.516	0.163	2.3	21.5	4.0
7	0.628	0.667	0.177	2.5	22.0	5.2
8	0.530	0.527	0.148	2.0	23.0	4.5
9	0.514	0.488	0.141	2.5	23.0	4.5
10	0.524	0.513	0.167	2.5	23.0	4.5
*11	0.612	0.631	0.178	2.5	23.0	4.5
12	0.554	0.551	0.159	2.0	22.0	4.5
13	0.531	0.539	0.162	2.0	22.5	5.0
14	0.534	0.517	0.154	2.0	22.5	4.5
15	0.530	0.524	0.171	2.0	21.5	5.0

With right vagus stimulation (figure 8), definite slowing occurred, but in a somewhat irregular manner. At the same time, no delay in conduction is apparent, but the striking diminution of the *P* wave, which nearly disappears, may have masked any lowering of conductivity. In those cycles showing almost an absence of the *P* waves, the *R* and *T* waves remain practically unchanged, so that it is the auricular portion of the electrocardiogram which is especially disturbed.

Left vagus stimulation (figure 9) produced only slight changes in the heart-beat in the one instance obtained. The only apparent influence on conduction is indicated by shortening of the *P–Q* time seen in the eighth and ninth beats, an effect exactly opposite to that seen in case I with left vagus pressure. No changes of note are seen in the *R* and *T* waves and only slight diminution occurs in several of the *P* waves.

The main interest in this case lies in the phenomenon of dissociation which was produced by right vagus pressure. It is seen in figure 10 that following vagus stimulation, a moderately prolonged cardiac cycle occurred, followed by a greater lengthening. At the end of this second lengthened cycle, 1.6 seconds after the initial pressure, there is a slight movement upward in the curve resembling the beginning of a *P* wave, which is interrupted by an entirely new type of ventricular complex consisting of a somewhat diminished *R* wave, a much exaggerated negative *S* wave, and a much enlarged *T* wave. This complex occurs in a series of three beats, each preceded closely by a small wave, evident from its position and shape as at least part of the *P* wave. The close proximity of the beginning of these *P* waves to the *R* waves makes it also evident that the auricles are not, during these three beats, the pace-maker for the ventricles. The abnormality of the ventricular complexes indicates that the stimulus of cardiac contraction does not pass through the ventricles in the usual way from the atrioventricular bundle, but that some abnormal point in the ventricles has taken up the pace-making function. The auricles and ventricles are beating

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independently, therefore, during the sixth, seventh, and eighth cycles, each portion of the heart at its own rate under the conditions produced by right vagus stimulation. From the measurements from this curve, it is seen that the ventricular rate is 78.5 per minute. Measurements from the rather poor brachial tracing and the electrical curve indicate that the length of systole is about the same, whether the ventricular beat is of normal or abnormal type.

FROM FIGURE 10.

Curve 41.3. Right Vagus Pressure. Dissociation.

Number of cycle.	*Q–Q.*	*P–P.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.502	0.502	0.165	2.5	22.0	4.5
2	0.490	0.480	0.165	2.5	21.0	4.5
3	0.494	0.485	0.175	2.5	21.0	4.5
*4	0.562	0.592	0.184	2.5	22.0	5.0
5	°0.753	0.881	0.154	1.5	——	5.0
6	°0.764	0.750	——	——	13.0	7.0
7	°0.776	0.714	——	1.0	13.0	7.0
8	0.668	0.629	—— (0.108)	1.0	14.0	7.0
*9	0.603	0.598	0.147	0.5	22.0	5.5
10	0.598	0.568	0.152	1.0	22.0	5.0
11	0.544	0.544	0.159	2.0	21.5	5.0
12	0.534	0.526	0.159	2.5	22.0	5.0
13	0.516	0.509	0.167	2.0	21.5	5.0
14	0.530	0.527	0.174	2.5	21.7	4.5
15	0.516	0.504	0.177	2.0	21.5	4.5
16	——	——	0.179	2.5	22.0	5.0

° = independent ventricular cycles. Independent ventricular rate = 78.5.

The brachial tracing gives no indication that these dissociated ventricular beats produce a weaker pulse than the others.

It should be noted that before the dissociation no appreciable delay in conduction is seen, while, when the auricles again resume their pace-making function, the conduction, as indicated by the *P–Q* time, is distinctly shortened, just as it is in figure 4. Here the flattening of the *P* waves, which is not seen in figure 4, may make this shortening only apparent, although it is quite reasonable to assume that the rest afforded the conducting system during the dissociation has rendered it capable of more rapid conduction than before.

The curves of especial interest from case III are those shown in figures 11 to 15. The undisturbed electrocardiogram (figure 11) is characterized by an especially prominent *T* wave. The heart rate calculated from the curve was 94.8 per minute, and a slight so-called sinus arhythmia is seen. The measurements average as follows:

$$R\text{–}R = 0.633, \quad P\text{–}R = 0.163, \quad P = 2.5, \quad R = 13.2, \quad T = 4.0.$$

Curves 66.2 and 66.4 (figures 12 and 13) demonstrate the difference in the effects of right and left vagus stimulation in this case.

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FROM FIGURE 12.

Curve 66.2. Right Vagus Pressure.

Number of cycle.	*R-R.*	*P-P.*	*P-R.*
1	0.586	0.576	0.186
*2	0.642	0.663	0.188
3	°1.000	1.229	0.180
4	0.966	0.730	——
5	0.574	0.578	0.177
6	0.556	0.550	0.170
*7	0.597	0.608	0.176
8	0.576	0.568	0.165
9	0.597	0.592	0.173
10	0.598	0.603	0.178
11	0.592	0.592	0.173
12	0.593	0.586	0.173

° = independent ventricular cycle. Independent ventricular rate = 60 per minute.

FROM FIGURE 13.

Curve 66.4. Left Vagus Pressure.

Number of cycle.	*R-R.*	*P-P.*	*P-R.*
1	——	——	——
2	0.526	0.523	0.190
3	0.530	0.536	0.193
4	0.550	0.550	0.189
*5	0.541	0.527	0.194
6	0.544	0.544	0.208
7	0.544	0.570	0.208
8	0.549	0.534	0.182
9	0.550	0.555	0.197
10	0.550	0.558	0.192
*11	0.550	0.551	0.184
12	0.556	0.559	0.183
13	0.574	0.560	0.180
14	0.558	0.562	0.194

Again the difference in the action of the right and left vagus appears. Stimulation of the right nerve has slowed the heart rate and not affected conductivity; stimulation of the left nerve has produced exactly the reverse effect, although the delay in conduction is slight. No striking changes are seen in the sizes of the various waves in either curve.

Dissociation of auricles and ventricles occurs in the right vagus pressure curve. The *P* wave of the fourth cycle follows the *R* wave closely, falling between it and the *T* wave, thus indicating that auricular and ventricular systole occurred synchronously. Except for the distortion which this coincidence causes, and a slight increase in the height of the *R* wave, the ventricular complex retains its usual form, and it may be inferred, therefore, that the stimulus of ventricular contraction was propagated through the ventricles from the atrio-ventricular bundle in the normal course. The independent ventricular systole

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occurs exactly one second after the preceding ventricular systole, showing an inherent ventricular rate of sixty per minute. The impairment of conductivity seen in the first part of the curve may be the result of a course of digitalis which had lasted nine days but had been discontinued five days before the curve was taken.

Another example of the difference in the effect of right and left vagus stimulation in this case is seen in curves 77.5 and 77.4 (figures 14 and 15).

FROM FIGURE 14.

Curve 77.5. Right Vagus Pressure.

Number of cycle.	*R-R.*	*P-P.*	*P-R.*	*R.*
1	0.526	0.557	—	9.0
2	0.529	0.517	0.163	9.0
3	0.501	0.507	0.175	9.3
*4	°1.081	1.389	0.169	9.0
5	1.060	1.175	—	10.0
6	0.475	1.144	—	10.0
7	°1.000	—	0.215	10.0
*8	0.988	0.894	—	9.5
9	0.697	0.684	0.142	9.0
10	0.626	0.624	0.155	9.0
11	0.562	0.562	0.157	9.0
12	0.547	0.530	0.174	9.0
13	0.539	0.537	0.176	9.0
14	0.526	0.525	0.175	9.0
15	0.537	0.544	0.168	9.0
16	0.549	0.557	0.160	9.0

° = independent ventricular cycles. Average length of independent ventricular beats = 1.047 seconds. Rate of independent ventricles = 57.3 per second.

FROM FIGURE 15.

Curve 77.4. Left Vagus Pressure.

Number of cycle.	*P-P.*	*P-R.*	*R.*
1	——	——	8.3
2	0.494	0.177	8.5
3	0.494	0.177	8.2
4	0.524	0.175	8.0
*5	0.528	0.180	8.0
6	0.542	0.187	8.2
7	0.558	0.185	8.0
8	0.545	0.172	8.3
9	0.554	0.180	9.3
10	0.580	0.197	8.7
11	0.584	0.184	9.0
12	0.548	0.163	9.0
*13	0.532	0.164	8.5
14	0.538	0.173	8.5
15	0.510	0.170	8.5
16	0.538	0.180	8.5
17	0.534	0.165	—

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Although the electrocardiograms are somewhat unsatisfactory, the accompanying cardiograms from the apex beat make the curves of interest. The exceptionally pronounced apex beat gave very perfect records through the usual funnel receiver, so that it seems justifiable to consider the sudden decrease in size of the cardiograms as evidence of weakened ventricular contraction. The modifying influence of respiratory movements was excluded, and the diminution in intensity of heart sounds and murmurs lent further justification to our interpretation of the small cardiograms. Although no attempt is made to express numerically the difference between the cardiograms in the two curves, it is seen in the curves that left vagus pressure has caused apparently a greater decrease in the force of ventricular contraction than right vagus pressure. Consideration must be given to the fact, however, that left vagus stimulation has caused almost no effect on rate in distinction to the marked slowing that followed pressure on the right nerve. This difference in rate may be responsible for the difference in the cardiograms, for the long diastoles following right vagus pressure would allow time for recovery of the depressed contractility of the ventricles, which could not take place with the slightly reduced rate following left vagus pressure. Again it is seen, as pointed out in curve 19.5 (figure 5), that the *R* wave is increased in height in those beats where the greatest reduction in size of the cardiograms occurs, especially with left vagus pressure.

In the curve showing the striking dissociation (figure 14), the ventricles take up their own inherent rhythm in three beats and show a ventricular rate of 57.3 beats a minute, nearly the same as that seen in the curve (figure 12) taken two weeks previously. The ventricular rhythm is broken into by one beat coming through from the sixth auricular systole. The point on the curve when this auricular contraction occurs cannot be determined with certainty, but it seems to be coincident with the *T* wave of the preceding cycle and is followed after a long conduction time by the ventricular contraction. The length of conduction here probably results from the auricular contraction occurring toward the end of the preceding ventricular refractory phase. The ventricles, therefore, could not respond immediately to the stimulus from the auricles. That this lengthening is not the direct result of vagus stimulation seems probable, too, when it is observed that immediately after the dissociation, the conduction time is strikingly shortened and remains so for several beats. The conduction time is, however, lengthened in the left vagus pressure curve with almost no slowing of rate.

The curves from case IV (figures 16, 17, and 18) show a somewhat different type of reaction of the heart to vagus stimulation. Clinically the case differed from the others in that the rate was slower, the heart-beat less forcible, and the difference between systolic and diastolic blood pressure not so great. In curve 38.3 (figure 16), it is seen that the undisturbed electrocardiogram from this case differs distinctly from those of the preceding cases. The rate (75.7 per minute) is slower, and the *P* and *T* waves are distinctly less prominent. The measurements average:

$$R\text{–}R = 0.792, \quad P\text{–}R = 0.167, \quad P = 1.4, \quad R = 17.0, \quad T = 2.1.$$

Right vagus pressure (figure 17) produced moderate slowing of the heart

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and slight lengthening of the conduction time, while the most striking feature of the curve is the great reduction and almost total disappearance of the *P* wave during the time of vagus stimulation. The *R* waves show a tendency to increase and the *T* waves to decrease in height during this time. After the removal of the pressure, the heart rate increases and the cardiac cycles become distinctly shorter than those seen before the pressure was made and than those in the curve unaffected by vagus stimulation (figure 16). At the same time, the *P* waves and the *T* waves are slightly higher than in the unaffected curve. This is seen especially in cycles 12 and 13, while in cycles 11 and 12 the *R* waves are slightly reduced.

FROM FIGURE 17.

Curve 38.4. Right Vagus Pressure.

Number of cycle.	*R-R.*	*P-R.*	*P.*	*R.*	*T.*
1	0.810	0.190	1.3	18.0	2.3
*2	1.264	0.178	1.0	18.0	2.0
3	1.200	0.184	?	18.3	1.0
4	1.257	0.192	?	19.0	2.0
5	1.306	0.203	?	18.0	2.0
6	1.069	0.193	?	19.0	2.0
*7	1.074	0.178	?	18.0	2.0
8	0.997	0.170	1.0	18.0	2.5
9	0.868	0.178	1.0	18.0	?
10	0.719	0.167	?	17.0	2.5
11	0.636	0.170	1.0	16.0	3.0
12	0.663	0.176	1.5	16.5	3.0.
13	0.647	0.173	1.5	18.0	3.0
14	—	0.176	—	—	—

FROM FIGURE 18.

Curve 38.5. Second Lead. Left Vagus Pressure.

Number of cycle.	*R-R.*	*P-R.*	*P.*	*R.*	*T.*
1	0.740	0.155	2.0	16.0	2.8
2	0.766	0.169	1.0	16.0	2.0
*3	1.103	0.161	1.5	16.0	2.3
4	1.273	0.197(?)	?	17.0	2.0
5	1.162	?	?	16.0	1.5
6	1.018	0.183	1.0	16.5	1.5
*7	1.017	0.196	0.5	16.5	2.0
8	0.868	0.193	1.0	16.0	2.0
9	0.856	0.175	1.0	16.5	1.5
10	0.469	0.180	1.0	16.0	—
11	0.952	—	—	21.5	—
12	0.836	0.183	1.5	16.5	1.5

Left vagus pressure (figure 18) produced almost similar effects. The slowing is not quite so great, while the delay in conduction of the heart-beat from auricles to ventricles is about the same. The striking flattening of the *P* wave and the tendency for the *R* wave to be increased and the *T* wave to be decreased

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in height are seen here also. After the removal of pressure, there is, however, no quickening of the heart rate and no increase in the *P* and *T* waves or decrease in the height of the *R* wave. Otherwise in this case in the curves presented and in several others, no definite difference could be made out in the effects from stimulation of the right and left vagus, although well marked effects were produced by pressure over each nerve.

DISCUSSION.

In the discussion of the curves that have been described, two features, the differences in the action of the right and left vagi and the significance of the dissociation of auricles and ventricles, demand special emphasis.

In two of these cases of so-called chronic endocarditis, a definite difference between the action of the two vagi has been constantly demonstrated. The control of rate predominated in the right vagus nerve, presumably through its inhibitory action on the normal pacemaker of the heart-beat, the sinus node. The control of conduction of the heart-beat from auricles to ventricles predominated in the left vagus nerve, presumably through its inhibitory action directly on the conducting system. It will be brought out later that the dissociations following right vagus stimulation depend primarily on the ability of this nerve to reduce auricular rate. The factor of heart rate must be reckoned with when the difference in the effect of the right and left vagi on conductivity is considered. The prolonged cardiac cycles resulting from right vagus stimulation afford an opportunity for recovery of the conductivity not given to the more rapidly beating heart when the left vagus is stimulated. An examination of the relation of the conduction times to the lengths of the preceding diastoles shows, however, that this difference in rate is at least only a contributing factor, and that the left nerve acts especially on conductivity.

There is sufficient evidence in the curves and in the auscultatory phenomena that accompanied the other vagus effects for the belief that in two of the cases vagus stimulation acted directly on the ventricles, causing a marked diminution in the force of their contractions. From the one case where a comparison of the effects of the two nerves on the contractility was made, no definite conclusions can be drawn as to their relative effectiveness, although

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each nerve apparently lessened definitely the ventricular contractions. This effect of vagus stimulation was not studied in the other cases of this series. The curves furnish no evidence as to changes in the excitability or tonus of the heart.

The heart seems to respond not infrequently more quickly to stimulation of the right than of the left vagus. Often the maximal effects occur in the first or second beat after right vagus pressure becomes effectual, while they occur several beats later with left vagus pressure.

The influence which stimulation of the vagi has on the various waves of the electrocardiogram shows also a difference in the action of the two nerves. The most constant change in the form of the electrocardiogram is the reduction in the size of the *P* wave, which sometimes entirely disappears. The *R* wave is usually increased in height, and the *T* wave may be increased a number of beats after vagus stimulation has been made, or, as in one case, diminished synchronously with the diminution of the *P* wave. The *P* waves, which represent the auricular activity, were practically always more affected by right vagus stimulation than by left, while in the cases where the *R* waves, which represent the initial activity in the ventricles, were increased, this change was greater after left than after right vagus pressure. The greatest changes in the *P* and *R* waves usually occurred in the longest cardiac cycle or in the following cycle. It is noteworthy that in each of the three curves in which the cardiogram is also shown (figures 5, 14, and 15), the *R* waves reach their greatest heights in the heart-beats in which the weakest ventricular contractions are seen. This fact indicates that an increase in the *R* wave in the electrocardiogram cannot be taken to mean an increase in the force of contraction of the ventricles. The observation agrees with that of Hering (2), who found that when pulsus alternans was experimentally produced in animals, the weaker ventricular contractions gave a larger *R* wave than did the stronger contractions.

The difference in the action of the two vagi have not been constantly demonstrable by the somewhat uncertain method of vagus stimulation which we have employed. In case IV, pressure on each nerve produced striking but practically similar effects, and in an-

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other case quite contrary results were obtained in the one comparison that was made of the difference in action of the two nerves. This patient was a man of twenty-nine years with chronic endocarditis. Left vagus pressure was followed by greater slowing of rate but by less delay in conduction than right vagus pressure. The effects of vagus pressure were comparatively slight in each instance. The significance of this finding is not clear, but it indicates that an investigation of many more patients is necessary to determine with what constancy differences in the action of the two vagi can be demonstrated in man by means of vagus pressure.

In our first communication, we described the effect of right vagus stimulation on hearts with auricular fibrillation, and showed that long ventricular pauses occurred without any appreciable change in the auricular fibrillation. This effect was attributed to a lowering of conductivity to such an extent that all the impulses which would ordinarily reach the ventricles from the fibrillating auricles were blocked. At first sight, it would seem that in these cases the right vagus was capable of very marked effect on the conductivity, but when it is remembered that a large majority of the rapidly formed impulses arising in the auricles are ordinarily blocked, it is reasonable to assume that complete blockage for several seconds may be produced by the slight further lowering of conductivity of which the right vagus is capable. No comparative studies of the two nerves were made in the fibrillating cases, so it is not possible to say whether stimulation of the left vagus could have been more effectual even than stimulation of the right. We wish to emphasize the fact, however, that in these cases with auricular fibrillation, marked effects of right vagus stimulation on the ventricular rate, probably due to changes in the conductivity of the heart, can apparently be demonstrated, while in them no opportunity is afforded for conclusions as to the influence of the nerve on the rhythmicity of the heart.

There are, therefore, facts which do not seem to agree entirely with the findings in the cases where differences in the two vagi are clearly demonstrated. However, it seems probable to us that usually in man, in healthy as well as in diseased hearts, the control of the rate of the heart-beat predominates in the right vagus, and

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control of stimulus conduction predominates in the left vagus, not because of any specific activity in the nerves themselves, but, as Dr. A. E. Cohn has suggested to us, on account of a possible difference in the anatomical distribution of the two nerves.[1] The recent work of Garrey (3) on the comparison of the action of the two vagi on the turtle's heart has added weight to the suggestion. Rothberger and Winterberg (4) have pointed out differences in the action of the two vagi in dogs similar to those we have described in man, and curves published by Hering (5) and Rihl (6) have also shown them.

In three cases, dissociation between the auricles and ventricles has occurred after stimulation of the right vagus nerve. In other words, following pressure over the right vagus, the auricular rate has been slowed and the ventricular contractions have ceased to follow normally the slowed auricular rhythm, but have established their own rhythm for one, two, or more beats. The contractions of the two parts of the heart have occurred almost synchronously, for the *P* and *R* waves nearly coincide, and the rate of the ventricles has been, at least in the first beat of the dissociations, faster than that of the auricles. The independent ventricular rates in these cases were from 57.3 to 78.5 beats per minute.

The usual cause of complete dissociation of auricles and ventricles is complete heart-block, when the normal mechanism for the conduction of the heart-beat is so impaired, usually by an anatomical lesion, that the ventricles receive no stimuli from the auricles, and so the ventricular rhythm is established independently of the auricular activity. The inherent rhythmicity of the ventricles determines then the rate of their contractions. This rate is, as a rule, in adults about thirty beats a minute, although faster ventricular rates have been observed, as in the cases of Wenckebach (7) and Windle (8), in which the ventricles beat forty-six and sixty-six times per minute respectively.

In our cases, impairment of conduction seems to play no part in the production of the dissociations, as there is but slight delay

[1] As this subject is discussed by Dr. Cohn in his paper in this number of the *Journal of Experimental Medicine*, we shall not go further into it. For the same reason, we shall not attempt to show extensively the relation of our work to the results which have been obtained experimentally on animals.

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in the conduction of the heart-beat from auricles to ventricles before the dissociation, a decrease in the conduction time immediately after, and no true blockage of the heart-beat is seen. It is the inherent high rate of ventricular rhythmicity in these cases which is the determining factor in the production of the auriculo-ventricular dissociations which follow right vagus stimulation. As soon as the vagus action slows the auricular rate below that at which the ventricles would contract by their own inherent rhythmicity, the ventricles cease to wait for the auricles as their pace-maker. They break through the pause, establish their own rhythm, and interrupt the normal course of the stimuli of the heart-beat. When vagus stimulation is discontinued, the auricular rate increases to a point where it is again sufficiently rapid to resume its pace-making function. An essential feature of the action of the right vagus in the production of these dissociations is its much greater inhibitory effect on the auricular rate relative to its effect on that of the ventricles, the rate of the inherent rhythmicity of the latter being apparently uninfluenced by stimulation of the right vagus nerve.

These patients in which dissociation followed right vagus stimulation had in common an abnormally rapid heart rate when at rest; an abnormally large pulse pressure occurred at least in two of the three cases; and in all, the heart action was thumping. The foregoing facts and the form of the undisturbed electrocardiograms from these cases have led us to the conclusion that the unusually high rate of the inherent ventricular rhythm, which has been the essential causative factor in the production of the dissociations, is the result of a hypertonus of the cardiac accelerator nerves. The undisturbed electrocardiograms from the cases showed very prominent *P* and *T* waves, resembling those obtained by Rothberger and Winterberg (9), after both vagi were cut. These authors conclude that the prominence of these waves in the electrocardiograms is typical of increased cardiac accelerator activity. The *P* and *T* waves are usually well marked in children, but in our cases a further abnormal exaggeration of them is seen. It is perhaps noteworthy that the fourth case, in which dissociation never followed vagus stimulation, also failed to show any of the signs which we have taken as indicative of accelerator over-action.

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The question of accelerator tone is one which has not been very generally considered and little is known as to its normal occurrence. Hunt (10), however, who investigated the question extensively in dogs, reached the conclusion that the most important function of the accelerators, and perhaps their only demonstrable one, was their tonic activity.. Hunt has shown that the accelerators not only stand in antagonism to the vagi, but that stimulation of the right accelerator has apparently more effect on the heart rate than left accelerator stimulation. Rothberger and Winterberg (4) have confirmed this finding, and they also show that left accelerator stimulation raises the power of stimulus formation in certain regions, especially in the node of Tawara and in the left auricle. This action of the left accelerator nerve on the lower points of stimulus formation could not be demonstrated, as a rule, until the activity of the normal pace-maker, the sinus node, was depressed. They found that this depression could be accomplished not only by cooling and scarification but by vagus stimulation as well. It appears from their work (9) that the right accelerator predominates over the sinus node in the right side of the heart, while the left accelerator exerts a stronger action over the heart in the region of the atrioventricular bundle. There is some reason to believe then that each accelerator antagonizes each vagus more or less separately, and that there is a certain anatomical division between the innervation of the right and left side of the heart. This is probably only relatively true, however, as the nerves of the right side go over functionally into the domain of the nerves of the left side, and *vice versa.*

Rothberger and Winterberg (4) have shown that after section of all the heart nerves in dogs, simultaneous stimulation of the right vagus and left accelerator results in the establishment of subauricular autonomy. This phenomenon depends upon the fact that the hyper-accelerated rhythmicity of the atrioventricular node exceeded the rhythmicity of the vagus-slowed auricles, and so ultimately the atrioventricular node set the pace for both auricles and ventricles. Thus a true nodal rhythm is produced. During the transition between this nodal type and the usual rhythm, there occurred a time when the sinus node set the pace for the auricles and the atrioventricular node set the pace for the ventricles. Electrocardiograms

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of this phenomenon in their dogs are very similar to those we obtained from our patients during the dissociations[2] described above. In our cases, we believe that both accelerators were in a state of increased activity or hypertonus. Although the left accelerator was exerting continuously its activity on the atrioventricular node, producing a heightened state of rhythmicity, this effect did not become apparent, for the right accelerator through its action on the sinus node sustained auricular rate at such a pace that ventricular autonomy could not assert itself. When, however, the action of the right accelerator was offset by stimulation of the right vagus, the rate of stimulus formation in the sinus node was reduced momentarily below that of the highly accelerated atrioventricular node. The atrioventricular node then asserted itself as the pace-maker of the ventricles, and complete dissociation resulted.

The form of the ventricular complexes in the electrocardiograms in two cases (Nos. I and III) is the same during the dissociation as when no dissociation exists. This indicates that in spite of the fact that the stimulus of the heart-beat no longer reaches the ventricles as a result of auricular contraction, it does arise in some point above the ventricles themselves. The stimulus reaches them and passes through them in a normal manner, for it is well known that stimuli arising in abnormal points in the ventricles, as in ventricular extrasystoles, give abnormal electrocardiograms. It seems probable that the point of origin of the ventricular stimuli is the atrioventricular node of Tawara. This node is considered on account of its specialized structure to have a higher rhythmicity than other points in the region of the ventricles, and Winterberg (11) has shown that electrical stimulation of the exposed heart in this region produces a normal ventricular complex in the electrocardiogram. In the third case (No. II), the independent ventricular complex in the electrocardiogram during the dissociation has an abnormal form, and here some other point in the ventricles has taken up the rhythm.

Left vagus pressure never produced dissociation in these cases, probably, on the one hand, because stimulation of this nerve never reduced the auricular rate sufficiently to allow the ventricular au-

[2] See especially figure 4*b*, *Arch. f. d. ges. Physiol.*, 1911, cxli, 355, and figure 1*e*, *ibid.*, 1910, cxxxv, 567.

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tonomy to assert itself. Possibly, on the other hand, stimulation of the left vagus directly offset the tonic action of the left accelerator and so prevented the onset of that increased ventricular rhythmicity which would find expression in dissociation. Some other factor seems to operate besides the delay of the auricular rate in allowing the ventricular autonomy to assert itself, for in the same case dissociation sometimes occurred after cardiac cycles which were shorter than some after which no dissociation took place. This added factor might be considered to be the mechanical stimulation by pressure of the accelerator fibers which so often run in the vagus nerve; the well known, long latent period of the accelerator nerves, however, makes it very unlikely that this is the case.

It is our belief that in these three cases of so-called chronic endocarditis in which right vagus stimulation produced a dissociation between auricles and ventricles, hypertonus of the accelerator nerves is a striking and important feature in the pathological physiology. It is this factor that we consider responsible for the apparent overaction of the heart, as evidenced by the rapid rate, violent contractions, and the large difference between the systolic and diastolic blood pressure, indicating an abnormally large cardiac output per beat. It is this factor also that gives these cases the somewhat distinctive form of electrocardiogram and raises the inherent rhythmicity of the ventricles to such a point that they assume their own rhythm, independently of the auricles as soon as the auricular beats are reduced to a rate which is not excessively slow. The fact that the same characteristics are seen in the electrocardiogram and that the same type of dissociation may appear when the accelerators of the dog's heart are stimulated electrically, adds weight to this idea.

It is, of course, impossible to say why the hearts of patients such as these, who suffer from chronic cardiac disease after rheumatism, should show evidence of overaction of the accelerator nerves. There may be an increased susceptibility of the heart to the action of these nerves, there may be an absolute increase in their tonic activity, or there may be a relative hypotonic condition of the vagus inhibitory mechanism which allows the usually active tone of the accelerators (if such exists) to be abnormally effectual. It is possible that this hypertonus or constant overaction of the accelerators of the heart

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represents a compensatory phenomenon which has gone beyond the limits of usefulness. On account of the valvular lesions, no doubt, it is necessary for the heart muscle to increase its work per beat and per minute in order to maintain the circulation properly, but the impression is gained by clinical examinations of these patients, that their hearts beat with unnecessary violence and rapidity and that there must be a constant fatiguing strain upon the myocardium. An increase of rest for the heart muscle by slowing the rate of its contractions is certainly an essential factor in restoring the compensation in many cases of heart disease. Besides rest in bed and such general measures as ice to the praecordium, there is often nothing of avail in reducing the heart rate, for in many cases digitalis and its allies fail, as do all other drugs and procedures, to produce any permanent benefit. If hypertonus of the accelerators is an important feature in the pathological physiology of these cases, as we believe it is, a rational therapy should be directed at lowering the activity of the cardiac accelerator nerves. This may require a new form of cardiac therapeutics.

SUMMARY.

The electrocardiographic records taken during vagus stimulation by pressure from children suffering with chronic heart disease have shown that the stimulation of the vagi in these cases is strikingly effectual. In some of the cases, a definite difference was demonstrated between the action of the right and left vagi. The control of the rate of the heart-beat seemed to predominate usually in the right vagus nerve, while the control of stimulus conduction from auricles to ventricles apparently predominated usually in the left vagus. This difference in the two nerves probably exists on account of the difference in their anatomical distribution, the right vagus going especially to that part of the heart which controls the rate of contraction, the sinus node above the right auricle, and the left vagus going especially to that part in which the conducting mechanism is found. Each nerve, however, has to a lesser degree the function which predominates in the other. The whole heart seems to respond, as a rule, more promptly to right than to left vagus pressure, and fairly constant differences have been seen in the effects which stimu-

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lation of each nerve has on the various waves of the electrocardiograms. The stimulation of each vagus may influence directly the contractions of the ventricles, causing great diminution in their force.

Right vagus stimulation was followed at times by a complete dissociation of auricles and ventricles. The auricular rhythm was slowed sufficiently at this time to allow the ventricles, whose inherent rhythmicity is apparently unaffected by right vagus stimulation, to take up their own independent rhythm. The heightened ventricular rhythmicity in these cases allowed this to take place after only moderate slowing of the auricles. The independent ventricular rhythm was sometimes established in the region of the node of Tawara, for no disturbance of the ventricular portion of the electrocardiogram occurred. At another time, some other point in the ventricles inaugurated the stimuli of the independent ventricular contractions and an abnormal electrocardiogram resulted.

The resemblance of our curves, showing dissociation, to those obtained during right vagus and left accelerator stimulation in dogs is definite. That analogy, the clinical picture, and the form of the electrocardiograms of these cases have led us to the belief that an important feature in the pathological physiology of these cases is hypertonus of the cardiac accelerator nerves. This factor, as a cause of symptoms and as a hindrance to the establishment of cardiac rest, may prove of great importance, against which a new form of cardiac therapeutics must be directed.

BIBLIOGRAPHY.

1. Robinson and Draper, *Jour. Exper. Med.*, 1911, xiv, 217.
2. Hering, Experimentelle Studien an Säugethieren über das Elektrocardiogramm, *Ztschr. f. exper. Path. u. Therap.*, 1909–10, vii, 363.
3. Garrey, Rhythmicity in the Turtle's Heart and Comparison of Action of the Two Vagi Nerves, *Am. Jour. Physiol.*, 1911, xxviii, 330.
4. Rothberger and Winterberg, Über die Beziehungen der Herznerven zur automatischen Reizerzeugung und zum plötzlichen Herztoden, *Arch. f. d. ges. Physiol.*, 1911, cxli, 343.
5. Hering, Experimentelle Studien an Säugethieren über das Elektrocardiogramm, *Arch. f. d. ges. Physiol.*, 1909, cxxvii, 155.
6. Rihl, Über Vaguswirkung auf die automatisch schlagenden Kammern des Säugetierherzens, *Arch. f. d. ges. Physiol.*, 1906, cxiv, 545.

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7. Wenckebach, Beiträge zur Kenntnis der menschlichen Herztätigkeit, *Arch. f. Anat. u. Physiol., Physiol. Abt.*, 1908, Suppl., 53.
8. Windle, Permanent Complete Heart-Block. A Case with an Exceptionally Frequent Ventricular Rate, *Heart*, 1910, ii, 102.
9. Rothberger and Winterberg, Über die Beziehungen der Herznerven zur Form der Elektrokardiogramms, *Arch. f. d. ges. Physiol.*, 1910, cxxxv, 506.
10. Hunt, Direct and Reflex Acceleration of the Mammalian Heart with Some Observations on the Relations of the Inhibitory and Accelerator Nerves, *Am. Jour. Physiol.*, 1899, ii, 395.
11. Winterberg, Das Elektrokardiogramm, seine theoretische und praktische Bedeutung, *Med. Klin.*, 1911, vii, 761 and 804.

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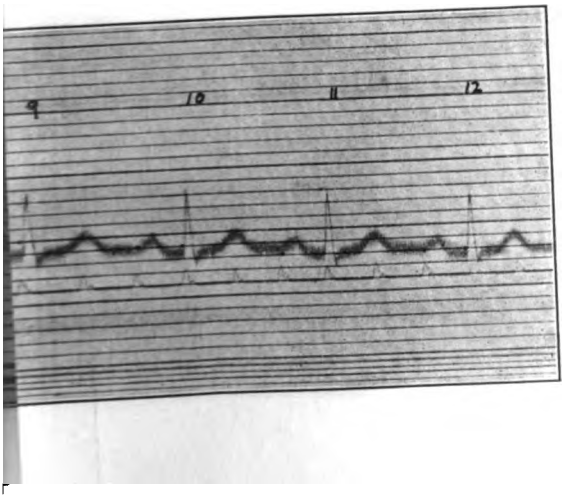
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PLATE 3

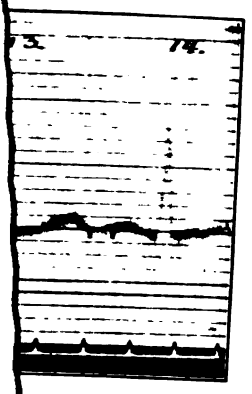


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PLATE 5.



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THE EFFECTS OF SPLENECTOMY UPON THE CELL CONTENT OF THE THORACIC DUCT LYMPH IN THE DOG, AND ITS RELATION TO THE ACTION OF PILOCARPIN.*

BY ROBERT L. DIXON, M.D.

(*From the Pathological Laboratory of the University of Michigan, Ann Arbor, Michigan.*)

In recent papers from this laboratory, references to which are given below, various observations on the cytology of the thoracic duct lymph under normal and under definite induced conditions have been made. To these reports are appended complete bibliographies of the subjects.

In anticipation of occasional reference to these papers in their relation to the work here reported, it seems desirable to summarize briefly in the words of the authors some of the points established.

"Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct" (Rous (1)).

"The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct" (Rous (2)).

Intramuscular injection of pilocarpin hydrochlorid is followed immediately by an increase in the number of cells in the thoracic duct lymph of the dog. "This increase is interrupted by the action of Roentgen irradiation, and the number can be reduced to below the initial count" (Dixon (3)).

In determining variations from relatively normal cell counts of the lymph, Rous's (4) data given in his paper, "Some Differential Counts of the Cells in the Lymph of the Dog," have been used.

In the course of these investigations, the relations which the spleen might bear to the processes involved and to the end results were considered to be of more than a passing interest. This was especially true in estimating the effects of pilocarpin on the lymph

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stream. The probable methods and seats of action by which lymphocytosis is induced by pilocarpin, have been cited and discussed in the papers referred to.

Previous to the reports cited above, the effects upon the blood of dogs of splenectomy and thoracic duct fistula, each alone and in combination, were observed and reported by Biedl and Decastello (5), Selinoff (6), Crescenzi (7), and Parodi (8), but the effects of splenectomy upon the cell content of the thoracic duct lymph have not been reported heretofore. Neither do I find discussed the results of splenectomy upon the effects of pilocarpin injections nor the effects of pilocarpin upon the results of splenectomy. These three points have been studied in the experiments to be described.

The accompanying tables are intended to be, to a great degree, self-explanatory, thus making unnecessary any detailed description of the results or of the steps in the various processes.

In all, ten experiments were made on ten animals. In each experiment, it was possible to obtain observations upon at least two of the points in question. The ten experiments will be taken up in the order in which they were carried out, and will be accompanied by such interpretations of the methods and results as seem warranted.

Dogs were used in all experiments. In every instance in which the animals were allowed to recover after the operation, as in cases of splenectomy to be followed later by an examination of the lymph, ether or chloroform was administered, and the operations were performed under regular surgical conditions. In the other cases, chloretone served as the anesthetic, and was administered in two gram doses either in alcoholic solution by means of a stomach tube, or, as was found to be equally effective and more convenient, in crystal form in one gram capsules. In all operations, care was taken to prevent any unnecessary hemorrhage. The exposure of the thoracic duct, and the insertion of the cannulas were done after the methods I have described in a former paper. Lymph smears and counts were made from drops as they appeared at the end of the cannula. These were stained by Wright's method, and by methylene-blue after fixation in absolute alcohol and ether. In only two instances was there any clotting within the cannula, and

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in both of these there had been a departure from our custom of keeping the cannulas in sulphuric acid and then washing them in distilled water.

The first three experiments were planned to demonstrate the late effects of splenectomy upon the lymph, also the effects of pilocarpin upon the lymph cell count a number of months after splenectomy. These three experiments are so much alike that they may be considered together.

At periods of eight, eight and one-half, and nine and one-half months, respectively, after splenectomy, thoracic duct fistulas were established and the routine examinations of the lymph and blood were made.

By reference to the tables, it is seen that twenty to thirty minutes after the insertion of the cannulas the total cell counts of the lymph cells in these three dogs were 7,850, 5,500, and 9,000 cells per cubic millimeter of lymph. In view of the fact that no counts

TABLE I.

Thoracic Duct Cannula Inserted Eight Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
20 minutes after insertion of thoracic duct cannula	7,850	427	46	8	481	17,000	110	420	530	
20 minutes after insertion of thoracic duct cannula										15 mg.
35 minutes after insertion of thoracic duct cannula	9,500	484	47	8	539	18,750	100	410	510	
35 minutes after insertion of thoracic duct cannula										30 mg.
55 minutes after insertion of thoracic duct cannula	9,900	430	29	9	468	22,500	91	370	461	

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TABLE II.

Thoracic Duct Cannula Inserted Eight and One-half Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	5,500	430	20	4	454	9,750	140	370	510	
30 minutes after insertion of thoracic duct cannula										15 mg.
45 minutes after insertion of thoracic duct cannula	6,200	420	22	8	450	10,750	130	382	512	
45 minutes after insertion of thoracic duct cannula										30 mg.
65 minutes after insertion of thoracic duct cannula	7,800	375	17	4	396	14,900	139	391	530	

of cells had been made before or at the time of the splenectomy, any change effected must be ascertained by comparison with counts made upon other animals.

By comparison with Rous's table (4) it appears that these counts, both total and differential, come well within normal limits. From this we are led to believe that upon the cell count splenectomy has no effect which is persistent through or after a period of from eight to nine and one-half months.

For the blood counts practically the same can be said. When we consider the wide range of variation in the total white cell count which might be brought about during the course of nine months by other factors than splenectomy, the differences here noted have little value. If counts had been made frequently during the interval, some significant change might have been discovered. Among the observers, however, on this point, there is a wide diversity of opinion.

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TABLE III.

Thoracic Duct Cannula Inserted Nine and One-half Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	9,000	270	16	5	291	12,000	110	238	348	
30 minutes after insertion of thoracic duct cannula										15 mg.
45 minutes after insertion of thoracic duct cannula	11,200	310	20	4	334	11,500	93	291	384	
45 minutes after insertion of thoracic duct cannula										30 mg.
65 minutes after insertion of thoracic duct cannula	12,700	460	34	7	501	14,800	121	406	527	

After material had been collected for these preliminary counts, an intramuscular injection of fifteen milligrams of pilocarpin hydrochlorid was administered. Fifteen minutes later, counts of cells showed an increase in the lymph and in the blood, except in the case of table III where there was a slight decrease in the total white blood cells. The increase in cells in the lymph is relatively greater than in the blood. Several counts were made during the next twenty minutes but showed no change beyond the usual variation in successive cell counts.

Fifteen minutes after the first administration of pilocarpin, a second quantity, this time thirty milligrams, was given in the same manner. Counts following this administration showed a further increase in the number of cells. The total increase secured by both doses of the drug varied between 1,000 and 2,000 for the lymph, and 2,000 and 5,200 for the blood. These were relatively

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large doses of pilocarpin. The increase secured is, at first consideration, somewhat marked, but upon comparison with the changes that pilocarpin induces in dogs that have not been previously treated as these animals had, it is relatively slight, for, in the control experiments cited, it was not uncommon to obtain an increase of more than 100 per cent.

We are led to think, then, that by removing the spleen, we have interfered with some important factor in the effects of pilocarpin upon the cells of the lymph and the blood. In addition to this, we may as reasonably think that some factor in the action of the drug remains which tends to increase the mononuclear cells of the lymph and both the mononuclear and the polymorphonuclear elements in the blood. A consideration of these factors will be taken up later.

At autopsy, these three dogs revealed nothing of note. The hemolymph-nodes could not be said to be increased in number or size. The effects in this respect are not at all comparable to the results of splenectomy in sheep, as reported by Professor Warthin (9).

In general, then, from these three cases we may say that: (1) splenectomy has no appreciable effect upon the cell content of the thoracic duct lymph after a period of eight or nine months; (2) the effect of pilocarpin upon dogs splenectomized eight or nine months previously, is a definite increase in the number of the cells in the thoracic duct lymph, but this increase is not so marked as in dogs which have not been splenectomized, as has been shown by former sets of investigations done in this laboratory.

The next three experiments were intended to exhibit the earlier effects of splenectomy upon the cell content of the duct and the action of pilocarpin. Accordingly, three dogs were splenectomized and then subjected to routine examinations for one, two, and four weeks, respectively. The conditions developing are shown in tables IV, V, and VI.

The comparison of the blood cell counts made at the time of the first and second operations, shows no uniformity in change. It will be seen by reference to tables IV, V, and VI, that two dogs underwent a decrease in white blood cells, and one showed an increase. The cases showing a decrease were examined one and two weeks, respectively, after splenectomy, while the case showing an

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increase was examined four weeks after the preliminary operation. From this we should not presume to conclude that the immediate

TABLE IV.

Thoracic Duct Cannula Inserted One Week after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						11,400	120	417	537	
30 minutes after insertion of thoracic duct cannula	4,300	364	30	2	396	9,500	74	221	295	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	6,800	471	42	6	519	14,900	114	362	476	

TABLE V.

Thoracic Duct Cannula Inserted Two Weeks after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclear,	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						20,250	110	420	530	
30 minutes after insertion of thoracic duct cannula	6,000	359	26	381	386	9,400	101	320	421	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	7,900	410	39	1	450	10,000	143	422	565	

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TABLE VI.

Thoracic Duct Cannula Inserted Four Weeks after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						13,500	66	217	283	
30 minutes after insertion of thoracic duct cannula	2,800	436	29	1	466	16,000	112	421	533	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	4,400	388	36	4	428	18,000	96	430	526	

effect of splenectomy is a reduction of the white cell count to be followed by a secondary rise after three or four weeks. Yet this is suggested when we take into consideration the fact that an increase in white cells in the blood is the usual sequence of an abdominal operation upon a dog. These decreases in cells would seem to be attributable to the removal of the spleen, and to be greater than the figures show on account of having to offset the usual rise in the cell count. To form any definite basis for such conclusions, it would have been necessary to make counts at frequent intervals, and to take into consideration the healing of the laparotomy wounds, the possibility of infection, the food furnished the animals, and all factors likely to effect changes in the cell counts.

On this point, observers have reached no uniform conclusion, for either their results have lacked uniformity or secondary factors were not taken into consideration in all cases.

On turning our attention to the lymph, we note by comparison with our adopted standards of normal counts, that the numbers fall within normal limits, but are relatively low.

When we compare tables IV, V, and VI, with tables I, II, and

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III, we find a definite relative decrease in the cells in the former, *i. e.*, in the animals splenectomized for only one to four weeks. In these, the average cell count is 3,000 or more per cubic millimeter less than in tables I, II, and III. It seems apparent, then, that the removal of the spleen establishes a lower cell count, but that this does not persist more than a few months.

In each of these cases, twenty milligrams of pilocarpin hydrochlorid were injected into the thigh muscles. Counts made thirty minutes later showed a definite rise in the number of both blood and lymph cells. These increases are practically the same in amount as in tables I, II, and III. But by comparison with the tables of dogs that had not been splenectomized, one must conclude that the effect is not marked. So it appears that soon after splenectomy—one to four weeks—the effect of pilocarpin is also much less than in non-splenectomized dogs.

In the next experiment, table VII, a cannula was inserted into the thoracic duct of a normal dog.

TABLE VII.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	2,500	320	30	7	357	
30 minutes after insertion of thoracic duct cannula						20 mg.
60 minutes after insertion of thoracic duct cannula	4,800	447	51	5	503	
60–65 minutes after insertion of thoracic duct cannula, spleen removed						
10 minutes after removal of spleen	5,250	241	10	1	252	
30 minutes after removal of spleen	3,000	310	16	4	330	

Thirty minutes after the insertion of the cannula into the thoracic duct, the cell count of the lymph was 2,500 per cubic millimeter. Twenty milligrams of pilocarpin hydrochlorid were then injected deeply into the thigh muscles. At the end of half an hour, the count had nearly doubled, reaching 4,800 per cubic millimeter. Then the

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spleen was quickly removed without loss of blood and with as little manipulation of the tissues as possible. This required about five minutes. The cell count was 5,250 per cubic millimeter, or more than 100 per cent. increase over the original count. Twenty minutes later the number had fallen to 3,000 per cubic millimeter, or to nearly the original number.

This experiment was planned to show the effect of splenectomy upon the changes induced by pilocarpin. From the figures, it appears that the pilocarpin brought about the usual increase of cells, which continued after the secondary operation, or was augmented thereby. Then, sooner than usual after this amount of the drug, the number of cells fell rapidly. It might be considered, therefore, that splenectomy inhibited in some degree the effects of the drug. This point will be discussed later in connection with other experiments made under equivalent conditions.

The first part of the next experiment, table VIII, is a duplicate of the preceding one.

TABLE VIII.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	4,000	218	31	3	252	
30 minutes after insertion of thoracic duct cannula						20 mg.
50 minutes after insertion of thoracic duct cannula	6,500	335	42	7	384	
50–55 minutes after insertion of thoracic duct cannula, spleen removed						
15 minutes after removal of spleen	7,100	360	35	4	399	
35 minutes after removal of spleen	3,400	240	22	4	266	
35 minutes after removal of spleen						20 mg.
55 minutes after removal of spleen	3,500	312	30	6	348	

The preliminary count of 4,000 per cubic millimeter increased in twenty minutes after administration of twenty milligrams of pilocarpin to 6,500. Then the spleen was removed, and a count taken fifteen minutes later showed 7,100 cells per cubic millimeter.

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Twenty minutes after this count, the number had fallen below the original count.

This again argues that by removing the spleen, we have interfered with the action of the pilocarpin, because, from former experience, we should not expect the effect of the drug to be exhausted so soon. However, it occurs that the point can be more definitely ascertained by administering a second dose of pilocarpin. Accordingly, a second dose of twenty milligrams was injected and the effects noted. After a period of twenty minutes, there was practically no change in the cell count, there being an increase of only one hundred cells per cubic millimeter.

This adds weight to the conclusion that the spleen is an important factor in bringing about the lymphocytosis induced by pilocarpin. The usual experience has been that a second administration approaches nearly the first count. In this instance, it is fair to say that the action of the drug was apparently inhibited by the absence of the spleen.

In the preceding experiments, we have shown by numbers the effects of splenectomy, both late and relatively early, upon the cell content of the thoracic duct. We have also demonstrated the effect of pilocarpin after splenectomy, and, in the last experiment, the effect of pilocarpin before and after splenectomy.

TABLE IX.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	8,600	291	41	0	332	
30–35 minutes after insertion of thoracic duct cannula, spleen removed						
10 minutes after removal of spleen	9,750					
20 minutes after removal of spleen	8,000					
30 minutes after removal of spleen	6,200					
30 minutes after removal of spleen						20 mg.
50 minutes after removal of spleen	7,800					
70 minutes after removal of spleen	5,000	310	43	4	357	

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The next step was to ascertain the immediate effect of splenectomy and how this effect is modified by pilocarpin.

The usual count was made of the cells in the thoracic duct lymph of a dog that had not been treated in any way. This count shows 8,600 per cubic millimeter. The spleen was then removed and the next count gave 9,750 cells per cubic millimeter, or an increase of over 1,000 cells per cubic millimeter. The next count, made ten minutes later, showed a decrease to 8,000 per cubic millimeter, and still later to 6,200 per cubic millimeter.

This then may be said to show that the early effect of splenectomy is an increase in cells in the lymph, followed very soon by a decrease which may carry the curve below the original count.

After making these observations, twenty milligrams of pilocarpin hydrochlorid were given, and at the end of twenty minutes the number had again gone up to 7,800 per cubic millimeter. This number was not maintained for any length of time, but in the next twenty minutes it had decreased to 5,000 per cubic millimeter. This action of pilocarpin is in harmony with the findings in similar circumstances in the preceding experiments.

In every case in which cell counts preceded and followed the operation of splenectomy, it appears that there is a definite rise in the number of cells immediately after the operation. This is true both with and without the association of pilocarpin. Although the operations were done with as little manipulation of the organs as possible, it was conceived that the mechanical factor involved in the handling of the spleen might to some degree be accountable for this effect.

It was determined therefore to massage the spleen in order to see if similar results would be obtained. After ascertaining the cell count of the lymph, the spleen was exposed and subjected to squeezing and manipulations. This was kept up for five minutes, after which the organ was dropped back into place.

The next count, ten minutes later, was as large as if a full dose of pilocarpin had been administered, for it showed 6,100 cells per cubic millimeter, whereas the original count was 3,800 cells per cubic millimeter. This is undoubtedly evidence that contractions of the spleen may be a great factor in increasing and in maintaining the cell content of the lymph.

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TABLE X.

Insertion of Thoracic Duct Cannula; Splenic Massage; Splenectomy.

	Lymph.	Pilocarpin hydrochlorid.
	Total cells per c.mm.	
30 minutes after insertion of thoracic duct cannula	3,800	
30–35 minutes after insertion of thoracic duct cannula, spleen exposed and massaged		
45 minutes after insertion of thoracic duct cannula	6,100	
55 minutes after insertion of thoracic duct cannula	5,000	
55 minutes after insertion of thoracic duct cannula		20 mg.
65 minutes after insertion of thoracic duct cannula	6,400	
75 minutes after insertion of thoracic duct cannula	7,000	
75–80 minutes after insertion of thoracic duct cannula, spleen removed		
10 minutes after removal of spleen	5,500	
10 minutes after removal of spleen		20 mg.
25 minutes after removal of spleen	5,000	

Ten minutes later the number of cells had fallen to 5,000 per cubic millimeter, and twenty milligrams of pilocarpin were given. The number of cells increased to 7,000 per cubic millimeter. The spleen was then removed, and the next count, ten minutes later, showed 5,500 per cubic millimeter. Another dose of pilocarpin was given, but it failed to increase the number of cells.

The general consideration of these experiments falls naturally under three heads: (1) the effects of splenectomy, immediate, early, and late, upon the thoracic duct cell content; (2) the effects of pilocarpin hydrochlorid upon the conditions existing after splenectomy; (3) the effects of splenectomy upon the conditions induced by pilocarpin hydrochlorid.

In addition to these three topics, we shall consider the factors involved in the action of pilocarpin.

(1) The lymphocytes in the blood after splenectomy have been counted and studied. Delamere (10) refers to the results of various workers and to the differences in their observations. He mentions the work of Uskow (11), Emilianoff (12), Hartmann (13), and Vaquez (14), who found the lymphocytes increased after splenectomy, and to Koroboff (15), who found them diminished. If we are to adopt the statement that most or perhaps all of the cells of this type in the blood are furnished it by way of the thoracic duct lymph, then we may assume that alterations in the lymph content

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will be manifested by changes in the blood content, and, on the other hand, that alterations in the blood content are consequences of alteration in the lymph content. These interdependent relations are found not to be constant, and should not be assumed to be so when we consider that lymph flow, cell destruction in the blood, and the ratio of the supply of these cells to their destruction in the blood, are not constant.

Yet it would appear reasonable to think that, accompanying or immediately preceding an increase in the lymphocytes in the blood, there is a relatively equal increase of these cells in the thoracic duct lymph.

By an examination of the tables, it appears that in all cases in which counts were made immediately before and after splenectomy, there is developed an increase in the number of these cells. This, however, is not permanent, and does not add to the sum total of the effect of pilocarpin. This is shown in tables VII, VIII, and IX. In tables VII and VIII, splenectomy was preceded by the administration of pilocarpin, and the increase might be construed as the further effect of the drug. But in table IX, splenectomy was not preceded by pilocarpin, and yet the increase is evident. This increase of short duration following each operation, gave rise to the thought that it might be due entirely to a mechanical factor; namely, the manipulation of the spleen and intestines. In experiment 10, the mechanical element was made prominent, and the results were very suggestive. An increase similar to that induced by pilocarpin occurred. If allowance is made for the time necessary for the duct to empty, we see that this increase was not persistent after the manipulation.

From these experiments I should conclude that the increase in lymphocytes in the blood and lymph immediately after an operation involving the handling of the spleen and intestines, is of purely mechanical origin. In conjunction with this, I may suggest that the action of pilocarpin in increasing the lymphocytes is mechanical, inducing contractions of the spleen and increasing the peristaltic movements of the intestines.

Furthermore, in experiment 10, it appears that the final removal of the spleen caused no increase in cells. This is not opposed to

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the view just stated, because in this case, the spleen had been subjected to vigorous massage and then to the action of twenty milligrams of pilocarpin. In that condition, it may be said to have been emptied of the cells which would have been expressed into the lymph circulation by the operation.

The early effect of splenectomy is ascertained only by a comparison with former general observations. With this in mind, we are led to believe that after intervals of one to four weeks, there is a relatively low cell content. This is probably to be explained on the basis of the absence of a white cell-forming organ. The first parts of tables IV, V, and VI give the data upon which this is based.

The late effect of splenectomy is to be seen in experiments 1, 2, and 3. As these counts fall well within normal limits, we must conclude that the operations had no lasting effects. To explain this, we may assume that there was a compensatory action on the part of other white cell-forming organs. The compensatory action of the hemolymph-nodes after splenectomy has been established by Dr. Warthin (9). In my cases, there was no such increase in the size and number of hemolymph-nodes as he secured in sheep. However, in two of the dogs, tables I and III, there were more and larger hemolymph-nodes than are usually found in these animals. In case 2, close search did not reveal any such nodes. It is perhaps significant that the lymph cell count in this dog was less than in the other two.

Another circumstance in this animal was very marked. Although dog 2 was operated upon at the same time with the others, and although its wounds healed perfectly, and it appeared to do equally well at first, it soon developed a marked degree of emaciation, and we found it imposible to improve his condition. Dogs 1 and 3, on the other hand, became quite fat and remained so during the time they were kept. Dog 2 was kept well supplied with food and water, and it was often noted that he devoured more food than the other dogs. He became vicious and killed several of the other dogs in his pen. Whether or not his inability to thrive on the food supplied to him was in any way related to the failure of the hemolymph-nodes to develop is not known. It is simply mentioned in this connection

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because the condition was strikingly different from that found in the other cases.

(2) The effects of pilocarpin hydrochlorid after splenectomy were demonstrated in all ten cases. With the single exception of the last, there developed an increase in the number of cells in the lymph. Tables I to IX show an immediate response to the action of pilocarpin. The striking feature of this increased number of cells is that the rise in the count did not nearly attain the height usually obtained by injections of pilocarpin in dogs not previously treated.

This was equally true in those cases in which, several months after splenectomy, the cell count had returned to normal. It is evident, then, that in estimating the importance of the spleen as a factor in the action of pilocarpin, the effects seen after splenectomy must be deducted from the total increase of cells in non-splenectomized animals.

We believe that the whole effect of pilocarpin in increasing the number of cells can be explained on a purely mechanical basis, as has been pointed out in the reports already cited from this laboratory. After one site of this mechanical action, together with an important source of the cells under consideration, is removed by splenectomy, we still have left regions in which this force is operative. Increased respiratory activity is an important factor that has been emphasized before. But there is another factor to which we find that attention has not been called, *i. e.*, the marked increase in peristaltic action of the intestines.

In the walls of the intestines and in the mesentery is situated a relatively broad channel of lymph-vessels. Under the increased activity of the musculature of the intestine, the flow of lymph is increased. At the same time, there is a great escape of fluid into the lumen of the intestine. As a consequence, not only is the flow of lymph increased, but the lymph itself, as well as the blood, undergoes condensation due to loss of fluid.

The deduction from the experiments, viewed in this relation, is that, after splenectomy, injection of pilocarpin causes an increase of cells in the thoracic duct lymph, which is to be explained by the increased activity of the respiration and especially of the coils of the intestines.

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(3) The effect of splenectomy upon the effects induced by pilocarpin is demonstrated in several cases. As has been pointed out, immediately after the operation there is an increased output of cells. This has been explained as being a consequence of the manipulation of the spleen. Very soon, however, there results a decided drop in the count, which we consider due to the removal of an important site of cell formation and of mechanical activity. Not all this decrease is to be attributed to the removal of the spleen, for we must realize that the other mechanical factors are gradually declining in their efficiency at the same time. It is evident, though, that removal of the spleen after the administration of pilocarpin hydrochlorid lessens materially the effect of the drug as measured by the number of cells in the thoracic duct lymph.

CONCLUSIONS.

1. Splenectomy in dogs results in an immediate increase of cells in the thoracic duct lymph, due to the manipulation involved in the operation. This increase is soon followed by a low cell count, attributable to the removal of an important source of the cells under consideration. Several months after the operation, a normal cell count is reëstablished, due probably to a readjustment of activity on the part of other cell-forming tissues.

2. Pilocarpin injections after splenectomy result in an increase of cells, attributable to an increased activity of the respiratory organs and of the intestine.

3. After the injection of pilocarpin, splenectomy may increase the cell count, but it causes a decrease sooner than in cases of pilocarpin injection not followed by splenectomy.

In the preparation of this work, I am under obligations to Professor Warthin for a general oversight of the problem; to Dr. Morse, of the Pathological Department, for much help in the routine of many of the experiments, and for making cell counts; and to Dr. Kollig and Mr. Hillman for assistance given several times in the splenectomy operations.

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BIBLIOGRAPHY.

1. Rous, *Jour. Exper. Med.*, 1908, x, 238.
2. Rous, *Jour. Exper. Med.*, 1908, x, 329.
3. Dixon, *Jour. Exper. Med.*, 1909, xi, 453.
4. Rous, *Jour. Exper. Med.*, 1908, x, 537.
5. Biedl and Decastello, *Arch. f. d. ges. Physiol.*, 1911, lxvi, 259.
6. Selinoff, *Arch. d. sc. biol.*, 1903, x, 273.
7. Crescenzi, *Fol. haemat.*, 1904, i, 418.
8. Parodi, *Arch. ital. d. biol.*, 1906, xlv, 258.
9. Warthin, *Jour. Med. Res.*, 1902, vii, 435.
10. Delamere, Poirier, and Cunéo, The Lymphatics, (translated by Leaf), Westminster, 1903.
11. Uskow, cited by Delamere, Poirier, and Cunéo, *loc. cit.*
12. Emilianoff, *ibid.*
13. Hartmann, *ibid.*
14. Vaquez, *ibid.*
15. Koroboff, *ibid.*

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CULTURAL STUDIES ON MOUTH SPIROCHÆTÆ (TREPONEMA MICRODENTIUM AND MACRODENTIUM).*

BY HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 7–11.

In the normal oral cavity of man and animals, three distinct types, at least, of spiral organisms have long been recognized. The first type is much larger and thicker than the other two and has only a few flat curves. It is known as *Spirochæta buccalis* (Cohn, 1875). The second and third types are more delicate in structure and possess a thin flexible body and quite regular, closely set shallow curves, the difference between the two being that one is smaller than the other. The smaller type is called *Spirochæta dentium* (Koch, 1877), while the medium form bears no particular name, but has been designated the "medium form" by Hoffmann and von Prowazek.[1]

The relative number of the three types of spirochætæ varies greatly according to the conditions of the mouth and to the localities from which the material is obtained. Thus, in my experience, the smallest type is more abundant in the deposit at the juncture between the teeth and the gum, and in the cavity of a carious tooth; while the medium and buccalis types are more frequently found in the mucus about the tonsils and pharynx, and in large numbers in ulcerative stomatitis.

As regards the pure cultivation of these organisms, our knowledge has been very meager. The only investigator who has succeeded in isolating them from associated bacteria in a culture is

* Received for publication, November 6, 1911.

[1] Hoffmann, E., and von Prowazek, S., Untersuchungen über die Balanitis und Mundspirochaten, *Centralbl. f. Bakteriol., 1te Abt.*, 1906, xli, 741, 817.

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Mühlens (1906).[2] The descriptions of his cultivated spirochætæ agree, in part, with the morphological features of *Spirochæta dentium* (Koch) and, in part, with those of the medium type. From this fact, Mühlens suggested that the medium type is nothing more than forms that, under certain conditions, spring out of the smaller type. However, he left this point unsettled. As the work to be presented in this paper shows, I was able to obtain at least two distinct types of mouth spirochætæ in pure cultures. One of them is the smaller type and has affinities with *Spirochæta dentium* (Koch), and the other belongs to the medium type. The morphological and cultural characteristics of these two organisms are so distinct that no confusion of the two should be made. It is not improbable that the culture of Mühlens contained both the smaller and the medium types. In fact, on two occasions, I have obtained such a mixed culture.

Hitherto, on account of the lack of pure cultures, a reliable means of differentiating various spirochætæ was impossible, and a loose terminology, such as has been in use for the spirochætæ of the mouth, was all that could be employed without risk. But, now that successful separation in pure cultures of morphologically distinct forms has been accomplished, it is possible to define two distinct species of mouth spirochætæ. I therefore propose the name of *Treponema microdentium* for the smaller, and *Treponema macrodentium* for the medium type. My reason for including these two types under the general specific name of "dentium" with the prefixes of "micro" and "macro" is that they belong unquestionably to the same genus, while the buccalis type belongs rather to another genus. In general characteristics, the micro- and macrodentium agree with the genus *Treponema* (Schaudinn) to which also the pallidum belongs.

CULTIVATION OF TREPONEMA MICRODENTIUM.

A small amount of tooth deposit, preferably from a young child, is put in a tube containing a large quantity of sheep serum water

[2] Mühlens, P., Ueber Züchtung von Zahnspirochäten und fusiformen Bacillen auf künstlichen (festen) Nährböden, *Deutsch. med. Wchnschr.*, 1906, xxxii, 797; Mühlens, P., and Hartmann, M., Ueber Bacillus fusiformis und Spirochæte dentium, *Ztschr. f. Hyg. u. Infectionskrankh.*, 1906–07, lv, 81.

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and a piece of sterile tissue (testicle or kidney of rabbit or sheep).[8] The culture medium thus inoculated is now covered with a layer of sterile paraffin oil. It is best to inoculate about three tubes which are incubated for about ten days at 37° C. During this time, the medium may become firmly or loosely coagulated through the action of bacteria simultaneously growing in the culture; a small amount of the culture is taken with a capillary pipette from the bottom of the tube and examined under the dark-field microscope. Numerous active small spirochætæ and other bacteria are usually seen. The next step is to purify the spirochætæ from the accompanying bacteria. The same principle is employed as for the purification of the pallida. For this purpose, a series of wide and tall tubes containing serum agar, in the ratio of 1 to 3, and a piece of sterile tissue (preferably sheep or rabbit) are inoculated with a capillary pipette with the impure fluid culture by inserting it carefully into the agar along the middle line until the point reaches the bottom of the tube. Then the contents of the pipette are gently forced in by means of a rubber bulb or a syringe, care being taken not to split the agar column by means of the compressed air. It is best not to empty the pipette within the agar, but to withdraw it with some of the contents still present. After withdrawal, the agar is covered with a layer of paraffin oil and the tubes are put in a thermostat. In ten days or two weeks, the tubes begin to show a hazy colony of spirochætæ spreading out from the stab canal that is now filled up with a whitish mass of bacterial colonies. When the size of the hazy colony becomes large enough to allow fishing, without risk of touching the stab canal, the tube is cut, the agar column split across very gently, and a sterile capillary pipette carefully inserted into the hazy zone from the clean newly cut surface of the medium. The material removed shows, under the dark-field, the presence of innumerable active spirochætæ. Pure cultures can then be obtained by inoculating a new medium with this colony by means of a capillary pipette. It may, of course, happen that purification may not at first be successful, so that it is necessary to repeat the same procedure until success is obtained.

[8] Noguchi, *Jour. Am. Med. Assn.*, 1911, lvii, 102; *Jour. Exper. Med.*, 1911, xiv, 99.

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CULTIVATION OF TREPONEMA MACRODENTIUM.

The same technique is used for isolating the microdentium. Special precautions must be taken (1) to obtain material in which the larger dentium organisms are predominating, as from a case of Vincent's angina or ulcerative stomatitis; (2) to enrich the larger type by several transplantations of the impure culture in fluid media; and (3) to select carefully the colonies that are characterized by fainter visibility and slower growth. The pure cultivation of the macrodentium is more tedious than that of the microdentium.

Sometimes it happens that the impure cultures show no, or only a slight coagulation of the serum constituents. In this instance, the purification is comparatively easy because the solid media do not become turbid and the delicate colonies of the spirochætæ radiating from the central stab canal can easily be recognized. But when the bacteria growing in the impure culture, as is the rule, produce acid and perhaps gas, the purification is rendered very difficult because the solid media become so turbid or torn up with gas that the colonies of the spirochætæ are not easily seen, or, when seen are difficult to isolate. Under such circumstances, it is advisable either to select better material for inoculation or to reduce the acid-producing bacteria by leaving the impure cultures for four or five weeks, during which time the spirochætæ remain alive and still transplantable while the bacteria often die. It may, however, be necessary to repeat this procedure several times before successful purification is attained.

Treponema Microdentium.—(Figures 1 to 10.) When cultivated in a serum agar tissue medium, a faint haze becomes perceptible within seventy-two hours at 37° C. along the stab canal. The density and dimensions of the hazy growth increase slowly for many days until the agar column two to three centimeters below the surface becomes diffusely hazy and opalescent. Along the stab canal, the growth becomes so dense that it appears as an irregularly thickened, whitish streak without definite contour. During the first and second weeks, numerous small spots of separate colonies scattered here and there appear around the stab canal, which are often seen to grow in the space between the agar column and the wall of the

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test tube. Young cultures have only a faint odor, but after about three weeks, the odor becomes characteristically fetid.

This spirochæta measures less than 0.25 of a micron at the middle of the body and gradually tapers towards both extremities, which are sharply pointed. The length varies according to the age of the culture: young cultures show many short forms, and old ones may reach eight microns in length and show, on the average, fourteen curves. The number of shallow and rectangular curves varies also according to the length of the specimen; but the angle and size of each curve are strikingly constant, although diminishing gradually towards the extremities. The pointed ends are usually drawn out straight along the hypothetical axis. The motion of the spirochætæ is quite active and rotating, and the body is flexible. Under certain conditions, a long thin flagella-like projection is observed at one or both ends. The general appearance of the organism is a straight line. When such a culture is disturbed by a capillary pipette or platinum loop, the regular straight forms become less evident and peculiarly twisted specimens more numerous. In these disturbed specimens, the curves, nevertheless, resemble the original. Singularly enough, these contracted individuals swim swiftly by a serpentine or vibratory motion. When the flagellum of one end is caught by a fixed object, the spirochæta swings actively as if attempting to liberate itself. Longitudinal division has frequently been observed in pure cultures.

As long as oxygen is completely excluded from a fluid medium, straight typical forms are produced; but when the medium is imperfectly anaerobic, the spirochætæ take on irregular and non-typical shapes.

The microdentium causes a loose coagulation of the serum constituents that renders litmus colorless after about two weeks' growth and the cultures exhale a strong fetid odor.

The injection of a culture of this organism into the testicles of rabbits and the subcutaneous tissue over the eye of a *Macacus rhesus* monkey, causes, within twenty-four hours, a marked induration that persists for about a week; however, no spirochætæ were detected in the inflamed tissue.

Treponema Macrodentium.—(Figures 11 to 16.) In the serum

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agar tissue medium, after about four or five days at 37° C., it grows as a faint hazy colony which is almost transparent and without demarcation towards the outside. Isolated colonies are rarely produced, and strict anaerobiosis is required for any growth to take place.

According to the condition of the culture, the morphological features of the macrodentium are subject to wider variations than those of the microdentium. In young cultures the organisms are plump and short with rather irregular shallow curves. The extremities taper off abruptly. Under the dark-field, the body of the organism shows double refraction; namely, a feeble line of refraction along the axis, bordered on both sides with stronger zones of refraction. The body is flexible and capable of creeping with great ease through the agar in all directions. When in a clear space, the organism swims freely with swinging or vigorously vibrating movements. Certain specimens have been observed in which the body shows a longitudinal cleft or division, and long delicate flagella-like projections, with minute curves, are attached to one or both ends. Specimens from an older culture are somewhat longer and thinner and taper more gradually towards the ends; the curves are almost rectangular, shallow, and quite regular; the body shows no longer double refraction, except near the middle. Motility seems to be lost in certain individuals, except for a few curves at the extremities that may still exhibit a swinging motion. Young organisms showing double refraction and having two to eight curves may measure 0.7 to 1.0 micron in width and 3 to 8 microns in length. Older specimens having fourteen or more curves measure usually 0.3 of a micron in width and 12 microns or more in length. The spirochætæ grown in a fluid medium show about the same dimensions, but many long specimens, corresponding to the length of several ordinary ones, may appear. Besides these, other individuals occur, the bodies of which are much heavier in the middle and show more closely set, regular, rectangular curves, and of which one fourth to one third of the body toward the extremities gradually tapers off to sharply drawn out points with comparatively few shallow curves.

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INVOLUTED FORMS.

I have already stated that when the exclusion of the oxygen is not complete, the microdentium produces many irregularly waved forms in a solid or fluid medium (figures 18 to 23). These individuals swim freely and are actively motile. By transplanting them into a new medium, under strict anaerobic conditions, they reproduce the normal forms. In fact, the forms which are commonly observed in a smear from tooth deposit consist of these irregular individuals; and it is only in an artificial medium, under strictly anaerobic conditions, that the regular straight forms showing a series of uniform curves appear. This fact is also true of the macrodentium.

On one occasion, I observed in an impure culture, in a fluid medium growing under imperfect anaerobic conditions, numerous spiral organisms with extremely regular, almost rectangular curves. Their length varied from four to sixty microns or more, and, with the corresponding number of curves, each averaged 1.8 microns. They were usually straight, finely tapered towards the ends, and apparently showed no real motility. Their bodies were flexible, but no modification of the form of the curves could be made out. The shorter ones, with few curves, are very thin (0.3 of a micron), while the longer specimens may be twice, three times, or four times as thick as the main portions of the body of the smaller varieties. Even with the longest forms, no cross septa, such as are easily recognized in certain other large spirochætæ, were seen. On the other hand, many larger forms showed a central cleft suggesting longitudinal division, which may run along part or the whole of the axis.

Transplantation of such cultures into a new fluid medium, kept under similar conditions of cultivation, causes these peculiar immobile spiral forms to reproduce indefinitely; while when cultivated in a solid medium under strictly anaerobic conditions they fail to grow at all.

Although I am inclined to view this finding as an example of involution of the macrodentium, I have not yet concluded whether we may not have before us rather a new spirochæta than merely an involuted form. The culture in which these spirochætæ appeared came from scrapings of an ulcer of the mouth of a child of three

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years of age, in whom ulceration, attended by fever, has been occurring regularly every three weeks since infancy. I am indebted to Dr. La Fetra for access to this case.

CONCLUSIONS.

I have described the successful cultivation of two distinct species of *Treponema* from the oral cavity of man. The species differ from one another morphologically and in respect to their cultural properties. As the two species have now been clearly separated and cultivated for the first time, I propose for them the names *Treponema microdentium* and *macrodentium*, respectively.

Division along the longitudinal axis has been demonstrated for both *Treponema macrodentium* and *microdentium*. When the micro- and macrodentium are cultivated under imperfect anaerobic conditions, they undergo involution, but still continue to vegetate to some extent. Finally, a spiral organism has been cultivated and described that may be merely an involuted form of the macrodentium, or it may be a new species altogether.

EXPLANATION OF PLATES.

PLATE 7.

FIGS. 1, 2, 5, and 6. *Treponema microdentium* in a pure fluid culture, twenty days at 37° C. × 1,400.

FIG. 3. *Treponema microdentium* in a pure solid culture, ten days at 37° C.; a few forms show longitudinal division.

FIG. 4. A preparation of the microdentium from a pure culture in a solid medium, five days at 37° C. × 1,400.

FIG. 5. An exceptionally long chain of the microdentium from a pure culture in a fluid medium.

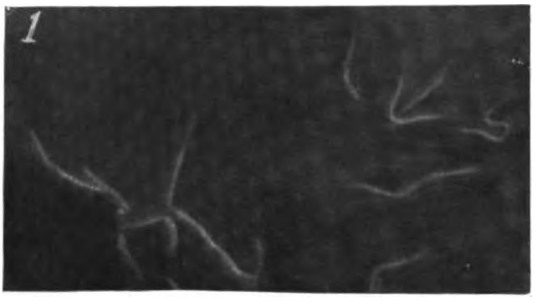
FIGS. 7 and 8. *Treponema microdentium* from a pure culture in a solid medium, twelve days at 37° C. Giemsa stain. × 1,400.

PLATE 8.

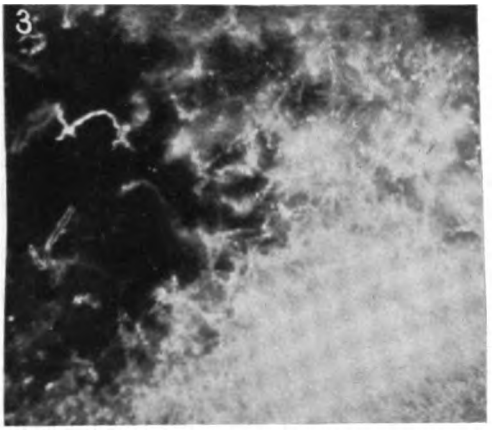
FIG. 9. A schematic reproduction of a dark-field view of the microdentium. A seven day old pure culture in a solid medium. × 1,400.

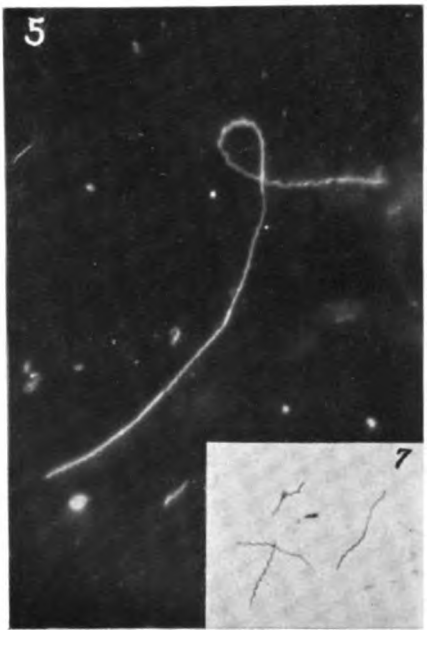
FIG. 10. The same as figure 8, but the culture has been disturbed by repeated punctures, and in this case the microdentium shows many involuted forms. × 1,400.

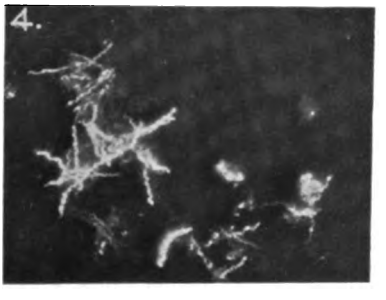
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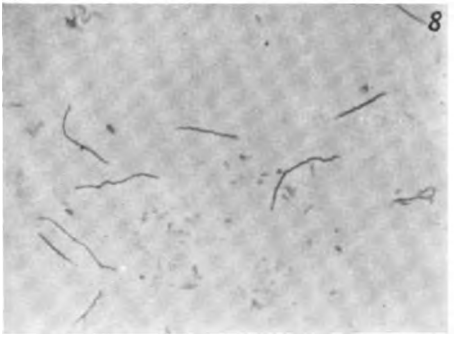






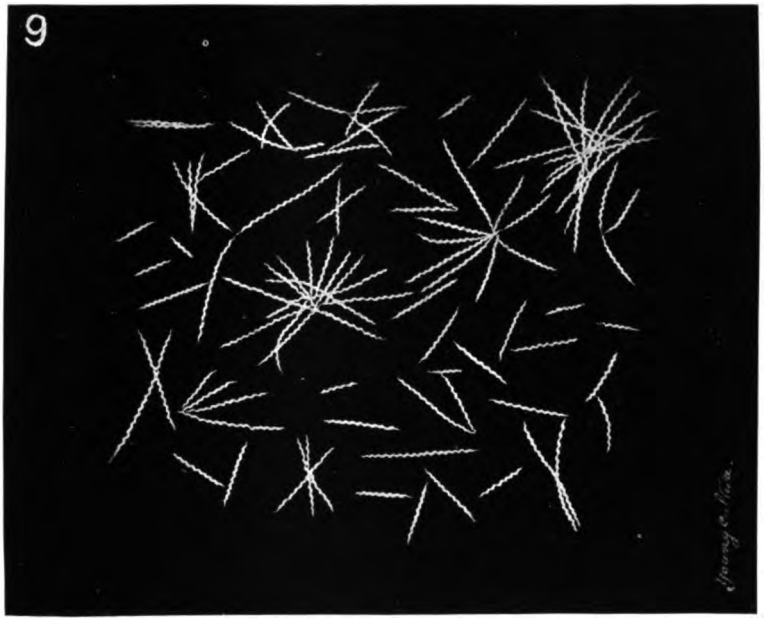


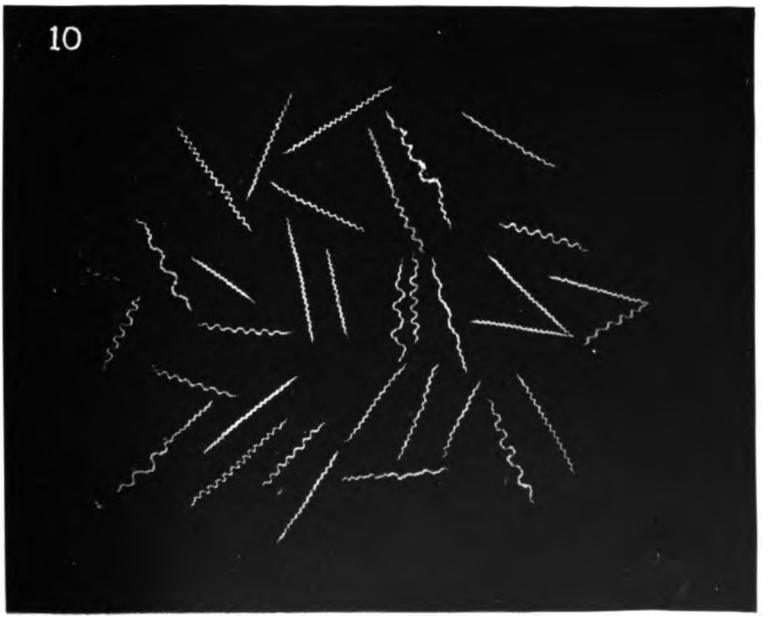




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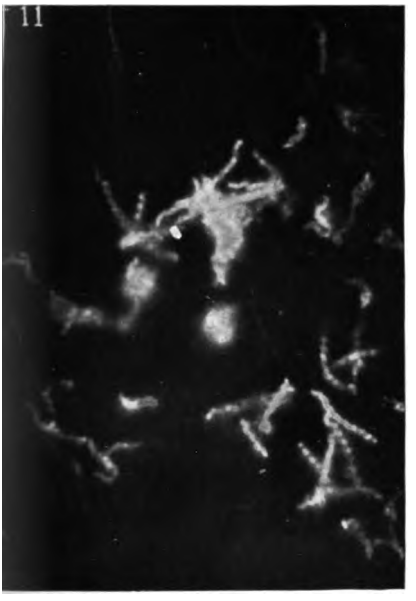
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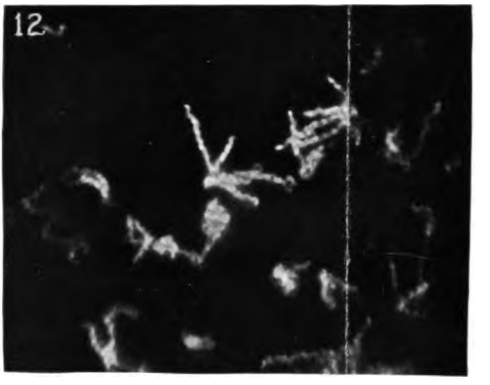




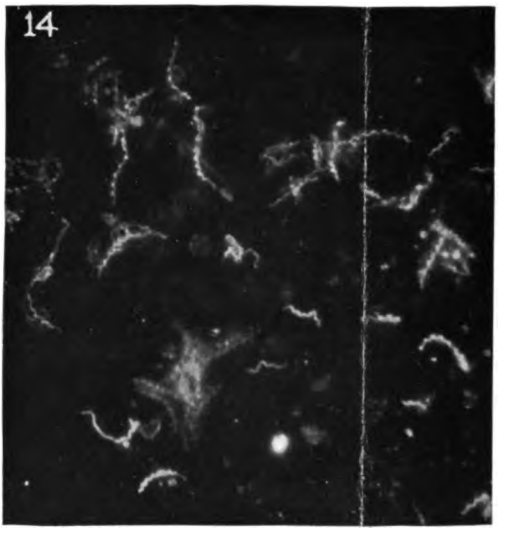
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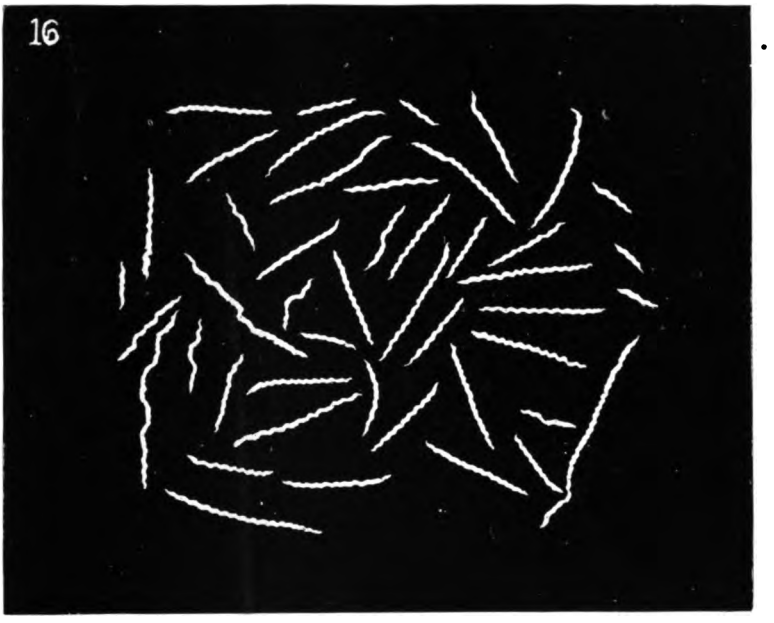


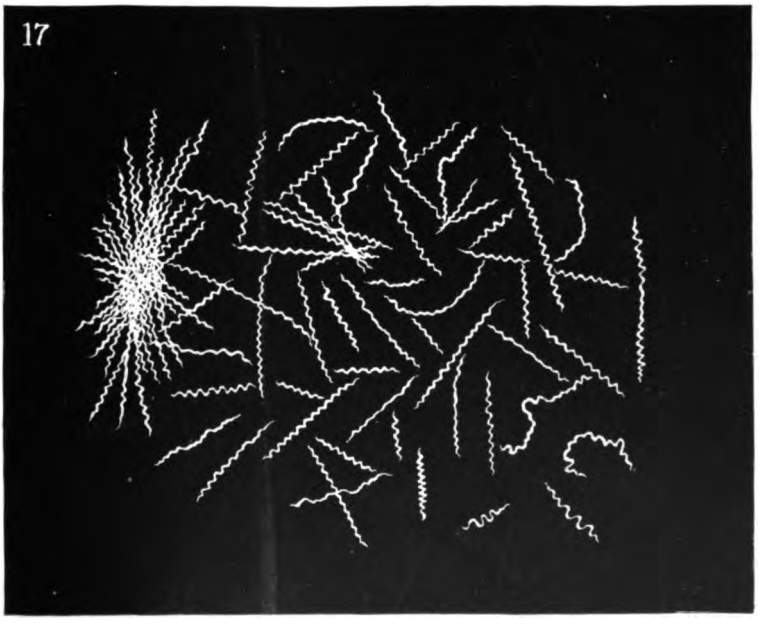




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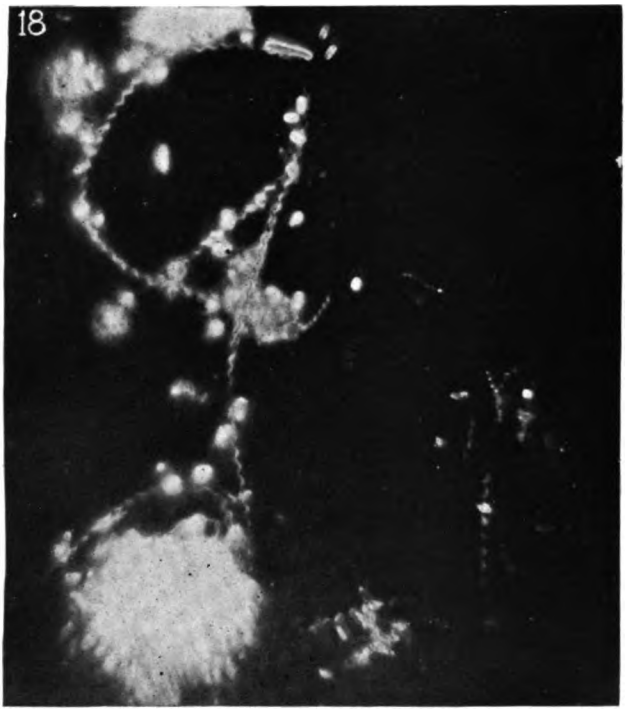
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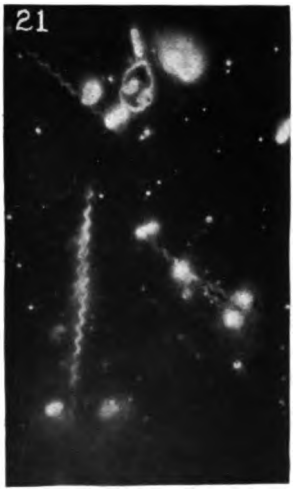


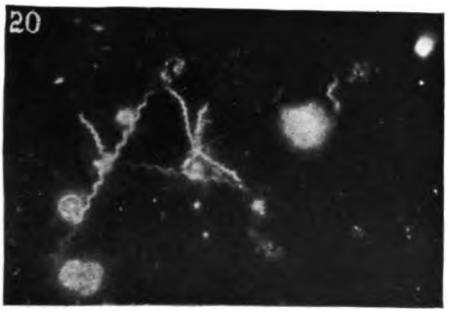
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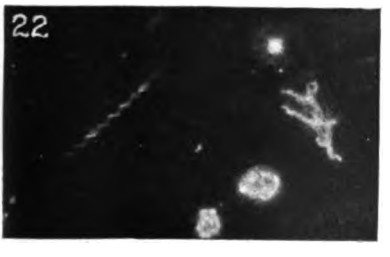
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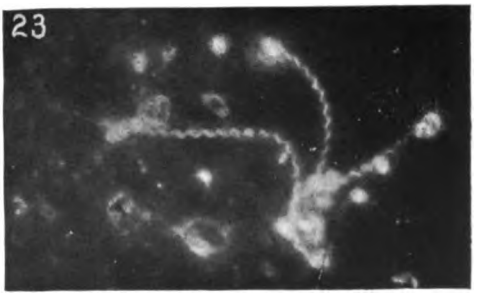












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PLATE 9.

FIGS. 11, 12, and 13. *Treponema macrodentium* from a young pure culture in a solid medium, seven days at 37° C. × 1,400.

FIGS. 14 and 15. The same from an older culture, many showing longitudinal division.

PLATE 10.

FIG. 16. A schematic reproduction of a dark-field view of a young solid culture (pure) of the macrodentium, ten days old at 37° C. × 1,400.

FIG. 17. *Treponema pallidum* from a pure solid medium, fourteen days old at 37° C. (For comparison.) × 1,400.

PLATE 11.

FIGS. 18, 19, 20, 21, 22, and 23. Involuted forms (?) of the macrodentium in an impure fluid culture. They show undeniable longitudinal division.

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THE DIRECT CULTIVATION OF TREPONEMA PALLIDUM PATHOGENIC FOR THE MONKEY

By HIDEYO NOGUCHI, M.D.

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THE DIRECT CULTIVATION OF TREPONEMA PALLIDUM PATHOGENIC FOR THE MONKEY.*

BY HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

PLATES 12–14.

I have described, in a previous communication,[1] a method for the cultivation of *Treponema pallidum* from the testicular lesions of rabbits inoculated with human syphilitic material containing that organism. At that time I had failed to cultivate the pallidum directly from syphilitic lesions in man, a feat that has now been accomplished. In cultivating the organisms from the testicular lesions, I found it advantageous to employ first a fluid medium and later, after adaptation had taken place, to substitute a solid medium. This is a satisfactory method for the testicular lesions which are nearly free from contaminating bacteria, but it is not applicable to the highly contaminated primary lesions in human cases of syphilis.

Two conditions are all-important in the working out of the method of direct cultivation: first, the maintenance of strict anaerobiosis; and second, the property possessed by the pallida of migrating in the solid medium in which they are multiplying. To meet these conditions, I employed a high cylindrical layer of a solid medium consisting of two parts of 2 per cent. slightly alkaline agar and one part of ascitic or hydrocele fluid, at the bottom of which has been placed a fragment of sterile tissue, such as rabbit kidney or testicle. I have concluded that the tissue acts chiefly by removing the traces of oxygen that exist in the lower levels of the medium, and probably also by providing a special form of nutriment. The solid medium, when inoculated with a fluid containing pallida and bacteria, seems to keep the latter fixed to the inoculation stab while

* Received for publication, November 25, 1911.

[1] Noguchi, *Jour. Exper. Med.*, 1911, xiv, 99.

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permitting the pallida to migrate to a distance where they may often be found in a pure or nearly pure state.

Comparisons have been made between the agar medium described and the gelatinized horse serum employed by Schereschewsky,[2] in favor of the former. The disadvantage of the gelatinized serum arises from the fact that the bacteria rapidly liquify it, and acids render it more permeable for bacteria. Hence it is very difficult to separate the spirochætæ from the contaminating bacteria.

DIRECT ISOLATION OF THE PALLIDUM.

Fifteen cubic centimeters of the medium described are placed into test-tubes measuring two by twenty centimeters. At the bottom is placed a fragment of sterile tissue. While rabbit kidney or testicle is preferable, human placenta, sheep testicle, or other sterile organ will suffice. The medium is prepared in the tubes, after the addition of the tissue, by mixing two parts of the melted agar at 50° C. and one part of ascitic or hydrocele fluid. After solidification, a layer of sterile paraffin oil three centimeters deep is added to prevent evaporation of the medium. A considerable number of tubes should be prepared at a time, and they should be incubated for a few days prior to inoculation, to determine sterility.

The material to be cultivated is obtained from lesions rich in the pallidum, such as chancre, condyloma, or skin papule, from which are snipped off, after cleaning with sterile salt solution, suitable fragments. These are immersed immediately into sterile salt solution containing in addition 1 per cent. sodium citrate. The fragment of tissue, which should be carefully preserved against drying, is then cut into small bits which are used as follows: one bit is emulsified in a mortar with the citrate solution; the others are inserted as such into tubes of culture medium, provided the emulsion shows, under the dark-field microscope, the presence of the pallidum.

Each of the half dozen bits of pallidum-containing tissue is inserted into a tube of the ascitic fluid tissue agar by being forced to the bottom by means of a thin blunt glass rod or heavy platinum loop, and into the same tube several drops of the emulsion are introduced by a capillary pipette also inserted deeply into the medium.

[2] Schereschewsky, *Deutsch. med. Wchnschr.*, 1909, xxxv, 835, 1260.

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Care is taken to avoid tearing the medium, and about six tubes are inoculated from each specimen of tissue.

The tubes should be incubated at 37° C. constantly for two to three weeks before examination.

The inoculated tubes usually become diffusely opalescent and show, as well, a dense opaque growth of bacteria along the stab-canal and about the wall of the tube. When considerable gas is formed, the medium is torn. The cultures are now punctured in several places with a capillary pipette which is inserted deeply into the tube, and the withdrawn contents are examined under the dark-field. The rule is to find large numbers of the pallidum imbedded in the agar and hence immotile. Should the organisms be present in a space clear of agar, they show active motility. Bacteria are always mingled at this stage with the pallidum.

The impure culture is fetid and putrefactive. Purification is begun by inoculating about twelve tubes with the impure culture by means of a charged capillary pipette that is carefully inserted in the centre of the agar column quite to the bottom of the tube. The contents of the pipette are expressed by means of compressed air, for which purpose I employ a syringe. The medium must not be split. The inoculated tubes are again incubated at 37° C. for two or three weeks. The bacteria grow in a dense mass along the stab-canal and on the surface of the medium. The tubes are inspected, from time to time, and in some a faint hazy zone will be seen to radiate from the stab-canal towards the sides of the tube. This hazy appearance is caused, usually, by masses of the pallidum that are growing away from the bacteria. When this growth has spread sufficiently far away from the stab-canal another transfer is made.

The surface of medium is sterilized with sublimate alcohol that is poured away. The tube is marked about the middle with a diamond pencil, and to the cut a pointed red-hot glass rod is applied. The tube cracks and the upper half is removed. The exposed surface of agar is sterilized with sublimate alcohol, after which the remaining moisture is wiped away with sterile absorbent gauze. By gently bending the agar column, it cracks transversely and exposes a clear surface on which the hazy growth is readily seen. Now, without touching the central canal, a capillary pipette is introduced into the

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haze and, after confirming, by dark-field examination, that it is caused by the presence of the pallidum, a series of inoculations are made in the manner described. It happens rarely that a pure culture is obtained on the first or second reinoculation; usually several reinoculations are required.

Not infrequently the growths of the pallidum are almost invisible. I am inclined to think that this is due to the quality of the culture medium. In this case, the dark-field microscope is the only reliable guide as to growth and situation in the culture of the pallidum. Again, the bacteria in the stab-canal may produce so much acid that it diffuses into the surrounding layer of medium where it causes a haziness capable of being mistaken for growth.

When ascitic or hydrocele fluid is not available, serum from the horse or sheep may be used for the purification process; they are, however, less satisfactory than the former fluids. It should be stated that I have found some specimens of ascitic fluid entirely unsuitable to the cultivation of the pallidum. When a favorable specimen has been obtained, it should be stored in sterile condition for future use.

PROPERTIES OF THE PURE CULTURES.

The pure cultures of the pallidum that I have studied have shown certain distinctive properties. First, the colonies of the pallidum that diffuse into the medium are never sharply, but always faintly visible, and the degree of visibility tends to be less in the pure than in the impure state. It seems to be unusual for the colonies to become discrete, although this has been observed when the sample of ascitic fluid has been very favorable. Second, the growth is most marked about the fragment of sterile tissue; I have not thus far succeeded in obtaining growth without fragments of tissue, although the medium used was similar in all other respects. In this important respect, the organisms that I have cultivated differ from those described by Mühlens[3] and by Hoffmann,[4] who obtained growth in a medium devoid of tissue. I have never observed my growths to cause any coagulation of the serum in a fluid medium, or to produce

[3] Mühlens, *Klin. Jahrb.*, 1910, xxiii, 339.

[4] Hoffmann, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1911, lxviii, 27.

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putrefactive odors, both of which are noted to occur by Mühlens and by Hoffmann.

When the medium is favorable, the first indications of growth appear in about seventy-two hours, and these increase gradually for two or three weeks, when the growth is at its height. The growth in a fluid medium takes place very slowly but continues for many weeks. The medium retains its fluid state indefinitely.

Morphology.—In young cultures, the greater part of the spirochætæ are short and actively motile. After growing for about two weeks, they attain the usual length of eight to twelve curves. Active motility is continued and the typical cork-screw movement is displayed. When the organisms are imbedded in the agar medium, they are immotilized. After several weeks, not only is the motility reduced, but the length has been increased (figures 1, 2, 3, and 5). The ends of the mature pallidum often show fine flagellum-like prolongations which are frequently perfectly curved, although sometimes they are devoid of curves (figure 6). The typical morphological characters are subject to disturbance by violent handling, as by the careless introduction into the growing mass of the capillary pipette or the presence of a trace of oxygen. Then the curves appear irregular, and straight forms occur. When irregular and atypical spirochætæ appear in certain tubes, they are due to the medium which is not strictly suitable, or to the presence of a trace of oxygen. To secure perfect and typical cultures, optimum conditions must be supplied.

Multiplication.—Schaudinn[5] described longitudinal division of the pallidum which he observed in two specimens. However, later investigators have described transverse division of the organism. It is important to ascertain which is the primary, or normal, and which the secondary, or abnormal, mode of division. That appearances suggesting both longitudinal splitting and transverse division are observable is admitted: the question relates to the significance of each process. Those who regard the longitudinal process as the normal one for multiplication look upon the transverse process as a last stage in the series of changes; while those who regard the transverse process as the normal one look upon the appearance of

[5] Schaudinn, *Arb. a. d. k. Gsndhtsamte.*, 1907, xxvi, 11.

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longitudinal splitting as caused by the coiling of one spiral organism, still united by a thin end filament, about another part of the same organism which is undergoing transverse division.

The disputed points relate to unstained and stained preparations directly obtained from lesions in man or animals in which the possibility of artefacts can hardly be removed. In both cases, the conditions are not distinctly favorable. What is demanded are periods of development of the pallidum during the stage of active division, and this is provided in the pure cultivation at certain stages of growth. I have now studied about ten strains at various stages of proliferation and growth and under varying conditions of cultivation, so that I have formed an objective conception of the process.

The mode of division of the pallidum in pure cultures is such as to admit of ready following of the phenomenon under the dark-field microscope. During the period of active proliferation, numerous dividing individuals may be seen in a single field. Several classes may be constructed according to the stage of the process (figures 7, 8, 9, and 10): (*a*) the body is divided at about two thirds of its entire length; (*b*) the body is completely divided and one part is wrapped about the other with regular curves or flat waves; (*c*) one half the body is divided and the other not, and the divided half has separated widely so as to produce a figure Y; (*d*) the partially divided body diverges at angles to produce V forms of varying width. When an acute angle is formed, tenuous protoplasmic bridges may span the interval; (*e*) two bodies remain united by a thin filament, one of which may be of normal or abnormal form; (*f*) three or more individuals may be united in a series of fine filaments suggesting imperfect transverse segmentation; (*g*) finally, two blunt wavy individuals each about double the thickness of an ordinary pallidum may remain together (figures 11, 12, 13, 14, and 15). This last form differs from the others in possessing a few granules that are definitely arranged and aggregated into bands that run across the organism. Whether this condition is degenerative remains to be determined. The longitudinal form of division is met with in all the cultures irrespective of the length of the spirochætæ. During the dividing process, the curves may be shallow and merely wavy; but after the separation is accomplished, the curves

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become deeper and regular. Motility is preserved during division, but is perhaps less active (figure 18, A, B, and C).

The various phases and varieties of the dividing process here described can best be followed when the spirochætæ are embedded at first in the agar medium that is pressed beneath the cover glass without disturbing their position. When the specimens are then liberated into a free space in which a fluid exists, the various phases can be readily traced as they pass from one to another, and in general in the order given, of the series described. Speaking broadly, the process is not rapid and requires about two hours for completion. The final result is two symmetrical halves, although asymmetrical division has been observed in rare instances.

PATHOGENIC PROPERTIES.

Treponema pallidum is a pathogenic organism. While its natural host is man, it is capable of implantation upon several higher animals, notably upon the monkey and the rabbit. I have already reported the successful inoculation of pure cultures of the pallidum, obtained from the testicular lesions of rabbits, to the testicles of normal rabbits.[6] Hoffmann[7] reported somewhat later the successful production of a specific orchitis by inoculating a pure culture derived immediately from human tissues. There is no real difference in these results, since originally all strains of the pallidum are human in origin. On the other hand, not all strains of the spirochætæ can be successfully implanted upon rabbits or other animals, when obtained directly from syphilitic lesions in man. Racial differences exist naturally, and probably can be produced artificially. The pallidum strains which I cultivated from the testicular lesions of rabbits and which were pathogenic for these animals, failed to produce skin lesions when inoculated into monkeys. But the strains described in this paper have been inoculated successfully into the skin of several monkeys.

In the experimental results to be reported, I employed three cultures of the pallidum derived from as many different human cases of syphilis and two species of lower monkeys, *Macacus rhesus* and

[6] Noguchi, *Jour. Am. Med. Assn.*, 1911, lvii, 102; *Jour. Exper. Med., loc. cit.*
[7] Hoffmann, *Deutsch. med. Wchnschr.*, 1911, xxxvii, 1546.

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Cercopithecus callitrichus. The inoculations were made by applying ten day old pure cultures in ascitic fluid tissue agar to the freshly shaven, scarified surface of the skin over the eyebrows, and, also, to the scarified prepuce, scrotum, or labia. The cultures were smeared over the prepared surface, and several small injections were made with a very fine syringe. The final results of the inoculations cannot be stated yet, but the present conditions are given in the following protocols.

PROTOCOLS.

Macacus rhesus, A.—October 2, 1911. A ten day old pure culture of pallidum, strain No. 7 (from a chancre), was inoculated to both eyebrows and the left labium.

October 9. The traumatic reactions have all disappeared.

October 15. Several small nodules appeared on both eyebrows, and a pea-sized induration at the site of inoculation on the labium.

October 22. The small nodules still persist, and in some there is an increase in size; some are scaly and congested. No pallida are seen in the expressed juice. The labium is still indurated.

October 29. Two distinctly indurated nodules on the left, and one small one on the right eyebrow. On examination of the expressed juice under the dark-field, some active pallida are found. The nodule on the labium shows no pallida.

November 6. The indurated nodules on the eyebrows show superficial ulceration. The expressed juice contains numerous active pallida. The labium is stiil indurated, but no pallida are found.

November 11. The size of the nodules on both brows is increasing, showing a moderate degree of congestion. The labium shows only a slight induration. Photographed. The blood gives a positive Wassermann reaction (figures 16 and 17).

Macacus rhesus, B.—October 2, 1911. Inoculation made as above with the same culture.

October 9. The traumatic reactions have disappeared.

October 15. Several small indurations on the eyebrows. The skin of the scrotum shows marked induration at the site of inoculation.

October 22. The size of the nodules is increasing.

October 25. Death due to pulmonary tuberculosis. The indurated tissues were excised and examined for the pallida, but none were found.

Macacus rhesus, C.—October 2, 1911. Inoculation made as in monkey B.

October 9. The traumatic reactions have disappeared.

October 15. One nodule on the left, and three small nodules on the right eyebrow. The labium is moderately indurated.

October 29. The nodules on the eyebrows have become larger and congested. The labium is indurated.

October 31. Death from pulmonary tuberculosis. Examination of the indurated tissues from the eyebrows shows numerous pallida.

Macacus rhesus, D.—October 2, 1911. A ten day old pure culture of pallidum, strain No. 8 (from a condyloma), was used for the inoculation.

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October 9. The traumatic reactions have all disappeared.

October 15. Several small indurations on both eyebrows. The labium shows marked induration at the site of inoculation.

October 22. No further changes.

October 29. The nodules are somewhat larger and fewer in number. The induration of the labium is still about the same. The expressed juice of the nodules on the brows shows a small number of active pallida.

November 11. The nodules are becoming smaller in size, and the labium is healing up. No pallida.

Macacus rhesus, E.—October 2, 1911. Inoculation as above.

October 9. All traumatic reactions have disappeared.

October 15. No change.

October 22. No induration.

October 29. No change.

November 11. Still no change.

Macacus rhesus, F.—October 2, 1911. Inoculation with a ten day old pure culture of pallidum, strain No. 9 (from a rectal condyloma).

October 9. The eyebrows are smooth and show no induration.

October 15. Several small indurations on the eyebrows.

October 22. The nodules are disappearing.

October 29. The nodules have disappeared.

November 10. No further change.

November 11. Death from pulmonary tuberculosis.

Cercopithecus callitrichus, G.—October 2, 1911. Inoculations with the same culture as in monkey F.

October 9. The eyebrows are clean.

October 15. There are several small nodules on both brows.

October 22. The nodules are increasing in size.

October 29. A distinctly raised nodule is seen near the corner of the left eyebrow. The small nodules are disappearing.

November 6. The nodule on the left brow has become very much congested and covered with a scaly crust. Under the crust, the nodule is barely covered with a new epidermis, which by slight scraping caused exudation of a clear serous fluid. Examination of the exudate showed a few active pallida.

November 11. The nodule is still much indurated and congested, but the surface is well covered. By gentle scraping, a serous exudate containing pallida escapes from the nodule. The blood gives a positive Wassermann reaction. Photographed.

From these results it can be inferred that pure cultures of *Treponema pallidum* are capable of causing, in certain lower species of the monkey, lesions of a characteristic form and history that resemble the lesions caused by inoculations of the spirochætæ-containing serum from a primary syphilitic lesion. Coincidentally, the development of the Wassermann reaction in the successfully inoculated monkeys has been observed.

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CONCLUSIONS.

A method for the direct cultivation of *Treponema pallidum* from human syphilitic lesions, by the employment of a solid medium, has been described. By means of it, three of the four strains worked with were successfully cultivated.

The several pure cultures agree in morphological and cultural characters, grow only in the presence of sterile tissue under anaerobic conditions, and do not produce putrefactive odors. The morphology is typical under optimum cultural conditions; it becomes atypical when the conditions are unfavorable.

In cultures, *Treponema pallidum* multiplies by longitudinal division. The process is usually symmetrical but occasionally appears to be asymmetrical.

Inoculation of the pure cultures into the skin of two species of lower monkeys was followed by the production of lesions resembling the primary syphilitic lesion occurring in man and those caused in the monkey by inoculation of spirochætæ-containing serum from human sources.

During the course of the positive inoculation in the monkey, the blood develops the property of giving a positive Wassermann reaction. Thus the relation of *Treponema pallidum* to this reaction is supported, and the identity of the cultivated strains with the species found in human syphilitic lesions established.

EXPLANATION OF PLATES.

PLATE 12.

FIG. 1. *Treponemata pallida* from a young pure culture in an ascitic agar tissue medium, four days old at 37° C. Dark-field. × 1,400.

FIGS. 2 and 3. The same after two weeks.

FIG. 4. *Treponemata pallida* from a chancre. (For comparison.) × 1,400.

FIG. 5. *Treponemata pallida* from a pure culture in an ascitic agar tissue medium, two weeks at 37° C. The pallida in free space show typical morphology.

FIG. 6. A short pallidum with flagella-like projections at both ends. × 1,400.

FIGS. 7, 8, 9, and 10. An ascitic agar tissue culture (pure) showing various phases of longitudinal division. × 1,400.

PLATE 13.

FIG. 11. A mass of dividing pallida from a pure culture in solid media. × 1,400.

FIGS. 12 and 13. Peculiar forms of dividing pallida from a pure culture in solid media. × 1,400.

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FIGS. 14 and 15. Dividing forms of pallida in fluid cultures.

FIG. 16. *Macacus rhesus, A,* with primary indurations on the skin above the eyebrows produced with a pure culture of the pallida. Photographed after forty days.

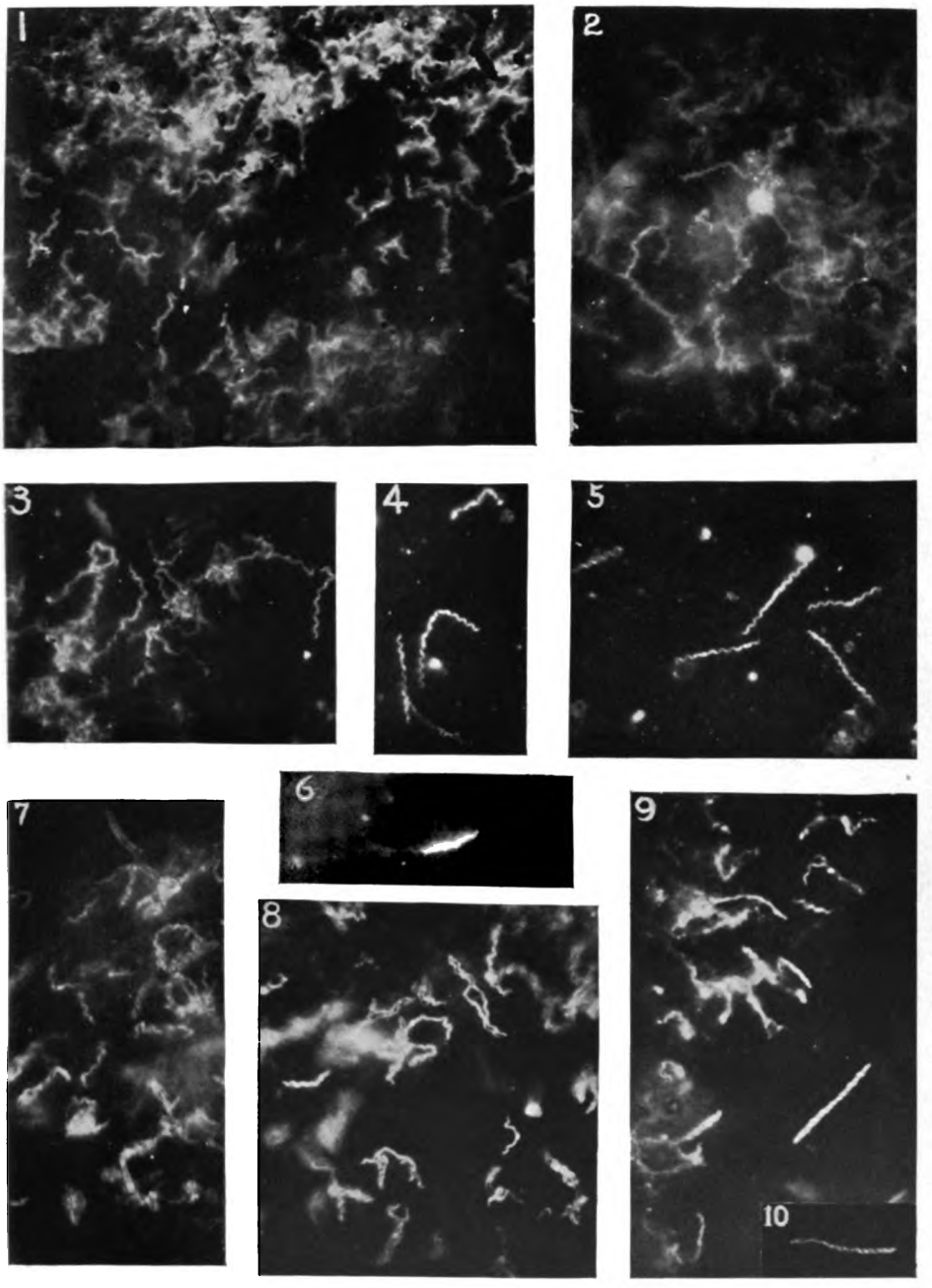
FIG. 17. A pallidum in the expressed juice of the lesions of the same monkey.

PLATE 14.

FIG. 18. A schematic reproduction of different phases of longitudinal division of *Treponemata pallida* in solid culture (pure). The drawings were made from observations on living organisms under the dark-field microscope. The description of these forms will be found in the text.

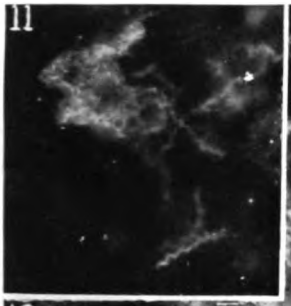
A = symmetrical division; *B* = asymmetrical division; *C* = irregular forms.

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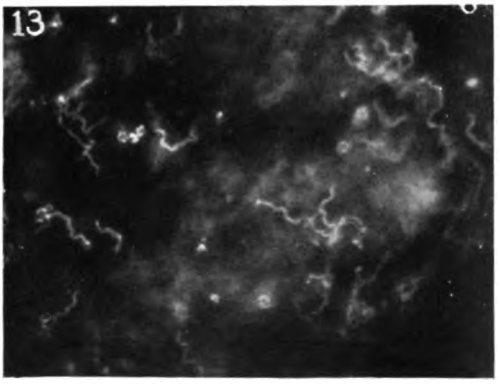


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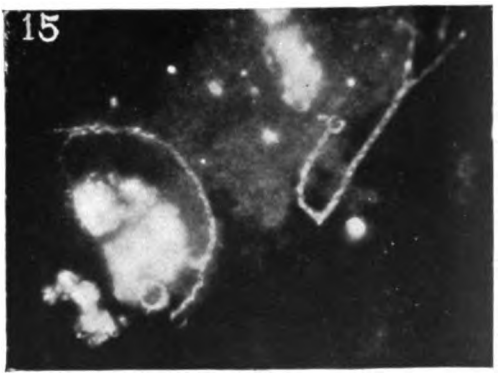
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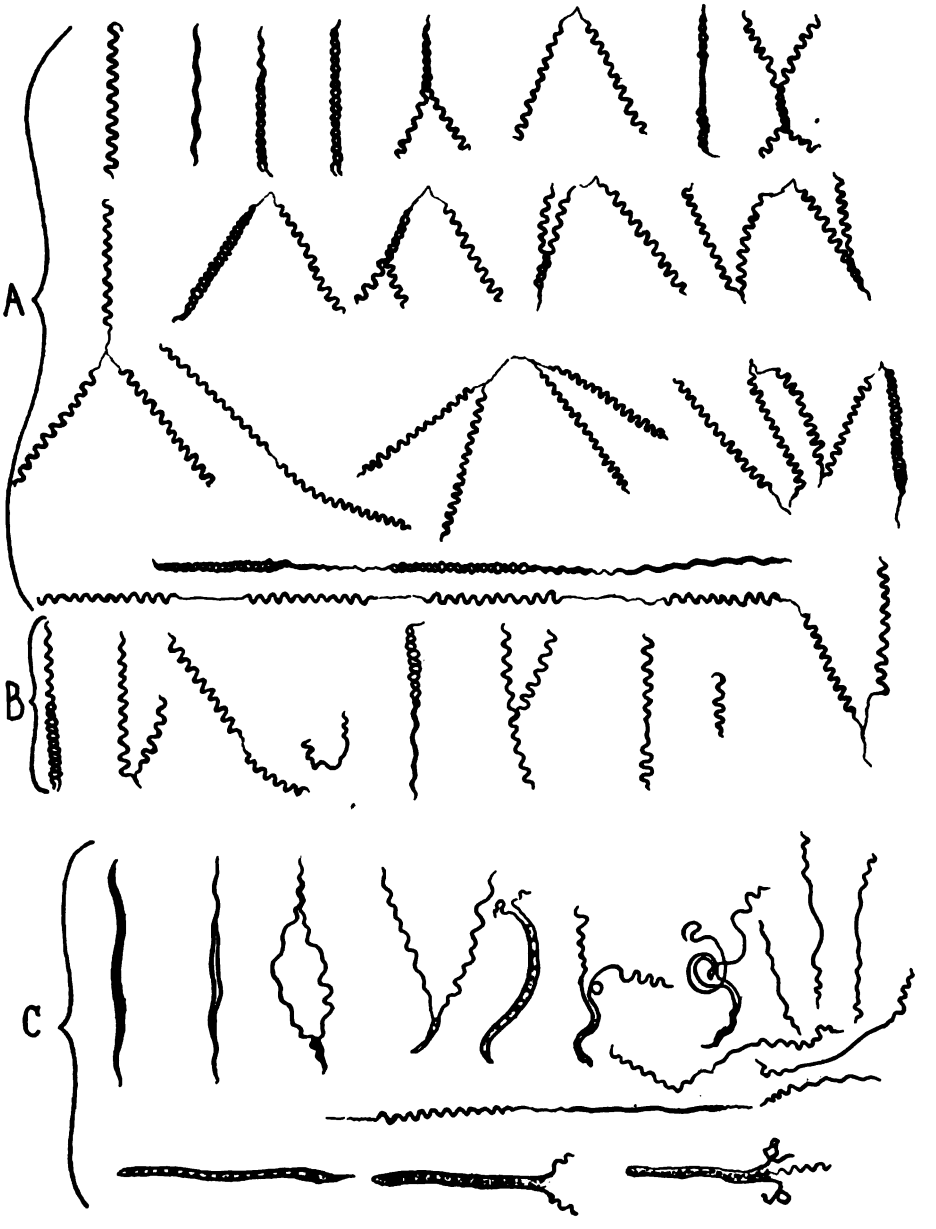






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STUDIES ON INFANT NUTRITION

UNDER THE DIRECTION OF L. EMMETT HOLT, M.D., AND P. A. LEVENE, M.D.

II. The Hard or Casein Curds in Infants' Stools *

ANGELIA M. COURTNEY
Fellow of the Rockefeller Institute New York

The casein curds in infant stools have been the subject of much discussion and controversy in recent years. Considerable disagreement exists in regard to both their chemical composition and the mechanism of their formation. All writers have been unanimous, however, in attributing to the curds much clinical significance, and in using them as a basis for choosing, or altering, the diet.

The older authorities regarded them as casein residues, appearing in the stools as the result of insufficient protein digestion and absorption. In recent years very emphatic protest against this view was expressed by Keller and Czerny.[1] According to their view, these curds were composed principally of a conglomeration of fat and soaps. Still more recently Biedert's[2] original view on the curds was revived, particularly by American writers. Selter,[3] Southworth and Schloss,[4] and Talbot[5] made chemical analyses of the curds and on the basis of his results reached the conviction that the principal component of these masses was undigested casein. The cause of their presence in the stools lay, according to these writers, in the disturbed protein digestion. Against this view there immediately arose opposition, led principally by the school of Finkelstein. L. Meyer and Leopold[6] did not deny the fact that the curds contained protein material, but, however, regarded the source of it not the casein of the ingested milk, but the intestinal secretions.

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*From the Laboratories of the Rockefeller Institute for Medical Research, and the Babies' Hospital, New York.

1. Czerny and Keller: Des Kindes Ernährung, etc., 1906, part 2, 17.

2. Biedert: Jahrb. f. Kinderh., 1881, xvii, 251; Kinderernährung im Säuglingsalter, etc., 1905, ed. 5.

3. Selter: Versamml. Deut. Naturforsch. u. Aerzte in Stuttgart in 1907, 177.

4. Southworth and Schloss: Arch. Pediat., 1909, xxvi, 4, 241.

5. Talbot: Boston Med. and Surg. Jour., 1908, clviii, No. 24, p. 905; 1909, clx, No. 1, p. 13; 1910, clxii, No. 5, 134; Arch. Pediat., 1909, xxvi, No. 12, p. 919.

6. Meyer, L. F., and Leopold: Arch. Ped., 1909, xxvi, No. 10, p. 773; 1910, xxvii, No. 2, p. 126.

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The cause of the appearance of the curds lay, according to the view of these writers, not in insufficient protein metabolism, but in faults of the carbohydrate assimilation and was brought about by excessive carbohydrate intake. These authors maintained that the appearance of the curds in the stools could be abated when the sugar intake was lowered.

Thus all writers are in agreement on one point, namely, that the presence of the hard curds in the stools is an indication of some fault of digestion, and that the health of the infant is in danger if the curds persist in the stools. The disagreements relate only to the interpretation of the nature of the disturbance and to the mode of treatment advocated by individual writers.

A mere superficial scrutiny of the literature on the subject reveals the fact that even the gravity of the symptom has never been firmly established; it was an assumption based on speculative considerations. Taking this assumption for granted, the individual observers applied their efforts in search for a method of treatment by which the symptom could be abated. The interpretation of the pathogenesis of the symptom was then determined by the success or failure of a certain mode of treatment. Thus, if the removal, complete or partial, of carbohydrate from the diet led to the disappearance of the curds, the theory was advanced that the disturbance of carbohydrate digestion was the basic cause of this symptom. Arguments of this nature can scarcely be regarded as very convincing. On the other hand, it was never clearly demonstrated that there existed a definite form of indigestion which invariably resulted in the curd formation in the stools, and still less evidence existed in favor of the assumption that the nutrition of the infants was affected severely in the periods when the stools contained the curds in abundance.

New experimental inquiry was needed in order to obtain reliable information on all these questions, and the present work was stimulated by such considerations.

Thus it is natural that the work should fall into two parts, one dealing with the relation of the curds to the state of digestion, and the second to that of nutrition. Information relating to the first part of the problem was sought on the grounds of the following reasoning: If the curds represent the remnants of undigested food, then their composition should be determined by the nature of the digestive insufficiency. Thus, if the curds consist principally of one component, of protein, of fat, or of carbohydrate, the conclusion would follow that there existed an insufficiency in the digestive capacity for that food-stuff. If that were so, one should also find that the composition of the entire stool showed a preponderance of that same component. On the other hand, should the curds have exactly the composition of normal stools differing only in the water content, the assumption may be justified that their formation

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is conditioned by irregularities of peristalsis. Finally the mass of the stools may, under some forms of indigestion, acquire such peculiarities of composition as would lead to the separation of one of its constituents in solid form. Thus, the accumulation of calcium salts may result in separation of lime-soaps; unusually high acidity may bring about precipitation of nucleins. Under such conditions one should expect the curds to have a composition distinctly different from that of the remainder of the stools, but would expect also a definite constant composition of the stool as a requirement for the curd formation.

From these considerations it is evident that it is not sufficient to limit the chemical investigation to the composition of the curds except when it is desired to establish the relationship between the state of digestion and the curd formation. On the contrary, it is very essential to supplement the analysis of the curds by that of the total stools. Furthermore, the insufficiency of the digestive organs may be essentially quantitative in its nature. That is, it may occur only after the intake of a certain constituent of the daily diet has overstepped a certain limit. Thus in order to be able to take into account all the factors engaged in the curd formation it is necessary to possess the knowledge of the chemical composition of the food, of the curds, and of the total stool in which they occur.

The data furnished by such analyses suffice to bring to light the bearing of any gastro-intestinal disturbance on curd formation, but they cannot help to interpret the influence of the curd formation on the state of general nutrition. It has been stated that there exists no experimental evidence in support of the view that the curds in infants' stools are indicative of some serious disturbance of nutrition. And yet the importance of the entire problem of curd formation depends on the answer to this question. In order to approach the solution of this problem the analyses of the food, of the curds, and of the total stools were supplemented by that of the urine, so that at least in regard to the nitrogen the exact daily balance could be established. Taking into account, in addition to this, the curve of the daily gain in weight, one can obtain fair information as to the general state of nutrition during the periods when the stools showed the greatest number of curds.

One other possibility needs to be analyzed before the discussion on the significance of the curds is exhausted, namely: Assuming that the curds represent a part of the ingested food which escaped digestion, one can easily recognize that the gravity of this condition would depend on the proportion of the food which escapes in that form. If the loss is considerable then normal nutrition could be maintained only by a corresponding increase in the daily diet. This information can be obtained by an estimation of the quantities of food elements contained in all the

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curds present in a twenty-four-hour sample of the stools. Naturally the curds have to be cleansed very carefully from the adhering feces. Such analyses were carried out in the course of the present investigation. They are recorded in Table 2.

Before passing to the report on the results of the present investigation it seems necessary to describe the general character of the curds, which are referred to in the present communication. It has been pointed out by several writers that much of the controversy in regard to the significance of the curds has resulted from the fact that writers were not sufficiently explicit in describing the appearance and the physical properties of the curds, and while some were dealing actually with fat-curds, other writers had under their observation curds of an entirely different composition. The curds referred to in the present paper possessed great similarity in their physical properties, though they differed considerably in their form.

Physical Character of the Curds.—Very commonly they consisted of rather hard, round or oval, not infrequently bean-shaped masses. Often they presented masses tapering at one end, or flattened into thin plates. The surface was generally smooth. The size varied from small fragments to masses more than an inch in length. The weight of such curds reached 2 gm. In several cases the combined curds obtained from a twenty-four-hour sample of feces (three or four large and two or three smaller masses) weighed between 7 and 8 gm. The surface was frequently stained deep yellow, while the inner part was usually white or cream-colored, more rarely yellow. In a few instances they presented a shining surface, persistent even after careful washing. Very often more or less numerous small fragments of the same material as the hard curds were found in the stools, either with or without the larger masses, possibly resulting from disintegration of the latter. These hard curds have the common characteristic of being more or less tough and resistant to pressure, and can thus be distinguished from the so-called fat-curds. The latter are generally soft and small in size.

On one occasion there was obtained a smooth, oval, moderately hard mass about 1¼ inches in length, which became liquid during the drying on a water-bath. On analysis it was found to contain over 80 per cent. of fat and between 1 and 2 per cent. nitrogen. Macroscopically it differed from the typical hard curds only in yielding more readily to pressure and in showing a smoother texture. The feces of the same child contained for a long period previous to that day the characteristic casein curds.

Chemical Composition.—Results of complete analysis of the curds are recorded by Selter[3] and by Talbot.[5] The total number of samples analyzed was eight, one by Selter and seven by Talbot. In regard to the protein content, there was an approximate uniformity in all the

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Number	Name	Date	Food Composition * Protein, Per Cent.	Fat, Per Cent.	Carbohydrate, Per Cent.	Nitrogen, gm.	Fat, gm.	Carbohydrate, gm.	Total Number Calories	Per Cent. of Dried Weight of Curds: Nitrogen	Protein	Fat	Ash	Description of Stools Containing Curds
1.	E. A.	Nov. 1910 5	1.75	...	5	3.02	....	54.0	334	9.8	61.2	20.0	...	Pasty; granular, yellow-green.
2.	A. P.	9	1.2	1.4	6	2.02	14.7	63.0	465	10.5	65.6	22.5	4.8	Pasty; granular, green-yellow, sour.
3.	S. R.	26	1.4	1.6	5.7	2.35	16.8	59.9	487	10.6	66.2	25.1	...	Loose; granular, green.
		Dec.												
4.	W. E.	7	2.3	.0	2.6	3.31	8.1	23.4	304	10.6	66.2	22.3	4.8	Loose-watery; granular, green.
5.	H. C.	7	1.8	.9	5	2.72	8.5	47.2	376	11.3	70.5	....	6.5	Pasty; granular, with mucus, green-yellow.
6.	H. S.	7	1.8	.0	2.25	3.03	9.5	23.6	307	10.6	66.2	19.0	4.8	Pasty; smooth-granular, green-yellow.
7.	P. C.	12	1.6	1.8	7.8	2.42	17.0	73.6	544	9.9	61.8	29.5	5.0	Pasty; smooth, pale yellow-green, sour.
8.	I. W.	16	1.4	1.6	5.7	2.35	16.8	59.0	487	9.5	59.3	....	...	Pasty; smooth-granular, yellow-green.
9.	T. S.	20	1.75	2.0	5	3.03	21.6	54.0	538	9.0	56.2	22.0	4.8	Pasty; smooth, yellow becoming green-brown, sour.
10.	T. S.	21	1.75	2.0	5	3.03	21.6	54.0	538	8.8	54.9	22.0	4.8	Loose-pasty; granular, green and yellow.
11.	T. B.	30	.4	1.6	5	2.35	16.8	62.5	459	10.1	63.1	....	4.8	Pasty; granular, yellow becoming green.
		Mar. 1911												
12.	C. R.	7-8	2.05	...	2.65	3.45	....	27.8	251	11.4	71.2	18.8	...	Loose; smooth, with mucus, yellow-brown becoming green.
										9.4	58.7	22.0	...	Pasty; smooth, pale gray-yellow, slightly green.

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Weight of	
Fat	Ash
9.3	24.6
8.9	20.3
9.9	16.0
5.7	23.8
3.3	20.0
9.3	23.0
6.1	24.4
3.2	28.5
2.6	27.8
3.3	28.9
1.3	15.9
9.3	16.3
1.1	14.3
5.6	20.8
4.7	15.5
7.8	15.2
4.5	13.8
5.7	21.7
0.7	27.0
0.4	20.9
9.4	20.7
5.2	20.1
4.0	27.9
6.0	23.0
8.9	20.2

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curds. Regarding the fat content Talbot recorded a considerable variation in different samples. According to this observer, the fat content the curds was determined by that of the food. In the course of the present observations more than thirty samples of curds were analyzed. Great care was taken to free the curds from adhering feces. This was accomplished by shaking them with water. The wash waters were removed by decantation, and the curds dried and used for analysis. The chemical analysis of these masses revealed a similarity in their composition which cannot be regarded as accidental. It is clearly seen from the tables that the protein, fat and ash content fluctuated within very narrow limits.

On the other hand, the composition of the stools was very variable, the nitrogen content fluctuating between 3, 4 and 5 per cent., only in one instance reaching 5.5 per cent., and in another 6 per cent.; the proportion of fat varied from 26 to 48 per cent., the ash between 20 and 31 per cent. The total acidity of the feces and the content of volatile acids also presented great variations.

Thus it is evident that the hard curds are composed differently than the mass of the stools and not infrequently contain more than 75 per cent. of protein. On this ground they cannot be regarded as masses of feces compressed into the peculiar formations owing to some irregularities of intestinal motility. They therefore represent either remnants of food that escaped normal digestion, or a normal component of the feces separated out of the mass of the stools owing to some peculiarity of the composition of the latter. The fact that the chemical nature of the feces and that contained the curds differed very markedly lends more support to the first of the two hypotheses, namely, that the curds represent remnants of undigested food, principally protein.

This naturally leads the inquiry into the causes of this partial indigestion. It has to be admitted that a very definite answer to this question cannot be given notwithstanding all the data furnished by the present work.

The great variations in the chemical composition of the stools which show the presence of curds argue against the assumption that there exists a definite form of indigestion which brings about the curd formation. To the same conclusion one is led by the analysis of the influence of diet upon the appearance of curds. It is clearly seen from Table 1 how varied the diet may be which renders possible the formation of curds in the feces. In still the impression is gained that the protein intake is a very significant factor in bringing about this symptom. Particularly the metabolism experiment on Daniel L. seems to furnish evidence in support of this view. This patient was under observation for considerable time and his diet was altered several times. It was observed that the curds

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appeared in the highest number and more continually when the percentage of protein intake reached the highest values. The only period when the stools were free from curds was when both the fat and protein intake were low and the carbohydrate intake comparatively high. The variation in fat intake alone remained without influence on the curd formation.

TABLE 3.—COMPOSITION OF STOOLS CONTAINING HARD CURDS—FECES WITHOUT THE CURDS

Name	Date	Total Nitrogen, gm.	Per Cent. of Dried Weight of Feces					Soap Fat. Per Cent. of Total Fat	C.c. N/10 NaOH Equivalent			
			Nitrogen	Protein	Fat	Soap Fat	Ash		Total Acidity of Feces	Volatile Acids of Feces	100 gm. Dried Weight Feces	
											Total Acidity	Acids Volatile
	Dec. 1910											
D. L.	6-7	.592	4.9	30.6	29.7	19.2	26.4	64.6	45.2	103.5	406	930
D. L.	7-8	.782	5.5	34.4	30.4	11.4	22.6	85.2	85.2	112.9	599	794
D. L.	12-13	.278	3.4	21.2	45.0	25.3	19.8	56.3	38.9	44.8	804	1,010
D. L.	13-14	.531	6.0	37.5	36.9	17.5	25.9	42.3	17.7	172.9	199	1,947
D. L.	15-16	.407	4.8	30.0	36.2	16.3	25.2	45.2	6.8	47.6	354	2,386
D. L.	17-18	.226	4.3	26.8	33.6	16.6	24.2	49.3	3.4	47.3	121	1,771
D. L.	18-19	.282	4.8	30.0	36.9	17.5	25.9	42.3	4.9	88.3	83	1,510
R. M.	14-15	.376	4.5	28.1	33.6	25.3	29.4	75.3	8.4	179.9	100	2,144
R. M.	16-17	.618	4.8	30.0	33.6	25.3	29.4	75.3	4.1	36.8	52	437
R. M.	18-19	.285	5.2	32.5	33.6	25.3	29.4	75.3	5.7	43.7	104	798
J. D.	7-8	.288	3.8	23.8	46.2	26.3	20.9	56.9	33.6	77.8	448	1,040
J. D.	9-10	.311	4.2	26.2	43.0	31.2	21.2	72.6	31.4	92.7	422	1,248
J. D.	12-13	.230	4.4	27.5	48.8	38.7	21.4	79.3	10.2	83.3	332	2,724
J. D.	14-15	.198	4.0	25.0	37.7	19.7	25.1	52.2	12.0	65.2	569	3,697
J. D.	15-16	.236	5.1	31.8	36.3	24.5	19.3	67.5	20.2	58.1	580	1,909
J. D.	17-18	.263	4.3	26.8	45.1	33.9	22.8	75.3	4.7	30.0	236	1,736
J. D.	18-19	.201	4.7	29.4	38.1	26.6	20.3	69.8	7.7	68.8	354	3,533
	March, 1911											
J. H.	13-14	.141	4.5	28.1	26.8	15.8	26.4	59.0				
J. H.	14-15	.164	4.3	26.8	32.4	19.2	31.2	59.3				
J. H.	17-18	.094	3.8	23.8	48.6	35.9	25.3	73.9				
J. H.	18-19	.170	4.1	25.6	31.5	13.2	22.5	41.9				
J. H.	19-20	.136	3.9	24.4	28.0	8.1	23.0	23.9				
J. H.	20-21	.245	3.8	23.8	35.4	16.7	28.3	47.2	17.5	44.8	270	693
J. H.	21-22	.247	3.3	20.6	48.3	5.0	24.2	10.4	23.1	72.4	311	976
J. H.	22-23	.123	3.7	23.1	39.8	15.9	20.6	39.9				

Thus it seems probable that under a variety of conditions, part of the protein intake escapes being digested and is removed by the stools in form of hard curds. The real mechanism of the curd formation is not easy to interpret for the reason that normally protein enters the intestinal canal from the stomach in a state of solution, and even if some of it enters in solid form the proteolytic enzymes of the pancreas are capable of bringing it into solution. If the protein is of the nature of a nuclein or paranuclein, as, for instance, casein, the alkalies present in the intestinal tract should be capable of effecting its solution. There is undoubt-

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edly a sufficient supply of proteolytic enzymes in the intestinal canal even under the conditions favorable to abundant curd formation, for the reason that often only a comparatively small part of the ingested protein appears in the stools in that form, the greatest part having apparently undergone normal digestion. Thus there must be some factors at play, which prevent the enzymes from penetrating the curd. No definite explanation of their nature can be offered at the present moment.

TABLE 4.—COMPOSITION OF STOOLS CONTAINING HARD CURDS—FECES INCLUDING THE CURDS

Name	Date	Feces. Dried Wt. in gm.		Total Nitrogen, gm.	Total Fat, gm.	Total Ash, gm.	Per Cent. of Dried Weight of Feces			
		With Curds	Without Curds				Nitrogen	Proteïn	Fat	Ash
	Dec. 1910									
D. L.	6-7	12.13	11.13	.682	3.53	2.99	5.6	35.0	29.3	24.6
D. L.	7-8	17.82	14.22	1.146	5.16	3.61	6.4	40.0	28.9	20.3
D. L.	12-13	11.20	8.35	.567	4.47	1.79	5.1	31.9	39.9	16.0
D. L.	13-14	9.88	8.88	.635	3.52	2.35	6.4	40.0	35.7	23.8
D. L.	15-16	11.18	8.38	.719	3.72	2.24	6.4	40.0	33.3	20.0
D. L.	17-18	6.02	5.32*	.235	2.37	1.39	3.9	24.4	39.3	23.0
D. L.	18-19	6.30	5.85	.331	2.27	1.53	5.3	33.1	36.1	24.4
R. M.	14-15	8.69	8.39	.411	2.88	2.48	4.7	29.4	33.2	28.5
R. M.	16-17	13.84	12.94	.716	4.52	3.85	5.2	32.5	32.6	27.8
R. M.	18-19	5.58	5.48	.296	1.86	1.62	5.3	33.1	33.3	28.9
J. D.	7-8	10.63	7.48	.619	4.41	1.70	5.8	36.2	41.3	15.9
J. D.	9-10	10.33	7.43	.603	4.07	1.69	5.8	36.2	39.3	16.3
J. D.	12-13	8.92	5.27	.625	3.67	1.28	7.0	43.7	41.1	14.3
J. D.	14-15	6.22	4.92	.347	2.21	1.30	5.6	35.0	35.6	20.8
J. D.	15-16	6.23	4.68	.399	2.16	.970	6.4	40.0	34.7	15.5
J. D.	17-18	10.98	6.33	.775	4.14	1.67	7.0	43.7	37.8	15.2
J. D.	18-19	6.67	4.12	.471	2.30	.920	7.1	44.4	34.5	13.8
	March, 1911									
J. H.	13-14	4.05	3.15	.225	1.042	.882	5.6	35.0	25.7	21.7
J. H.	14-15	4.55	3.80	.239	1.396	1.225	5.3	33.1	30.7	27.0
J. H.	17-18	3.20	2.50	.161	1.295	.670	5.0	31.2	40.4	20.9
J. H.	18-19	4.70	4.20	.217	1.380	.973	4.6	28.8	29.4	20.7
J. H.	19-20	4.20	3.50	.201	1.057	.844	4.8	30.0	25.2	20.1
J. H.	20-21	6.88	6.48	.282	2.340	1.853	4.1	25.6	34.0	27.9
J. H.	21-22	7.91	7.41	.291	3.632	1.818	3.7	23.1	46.0	23.0
J. H.	22-23	3.45	3.35	.132	1.343	.696	3.8	23.8	38.9	20.2

*Fat lump only separated, few small hard curds left in the feces.

It remains to discuss the data regarding the practical significance of the symptom. It has been stated before that the curds represent part of the food that has escaped being digested. It naturally follows that the injury occasioned by it to the economy of the organism may be very perceptible when the proportion of the non-utilized fraction reaches a high value. The review of Table 2 clearly demonstrates that such may be the case only on very rare occasions. Thus it is seen that out of seventeen stools, in nine the curds contained less than 1 gm. and not more

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than 1.5 gm. of dry material. Calculating their protein content at 65 per cent., the loss of protein occasioned by the curd formation in these nine instances ranged between 0.06 and 0.95 gm. in twenty-four hours. In four stools the total dry weight of the curds reached from 2.0 to 3.0 gm. This resulted in a protein loss varying between 1.35 and 2.0 gm. in twenty-four hours. In four other stools the dry weight of the curds was between 3.15 and 4.65 gm., which meant a loss of protein of from 2 to 3 gm. Calculating the twenty-four-hour intake at 20 gm., it becomes evident that only on very few occasions was the loss resulting from the curd formation considerable. Generally, however, the appearance of curds in stools in itself does not lead to a perceptible decline in the utilization of the food-stuffs.

Furthermore, this conclusion is corroborated by the metabolism experiments. It is seen from Table 5 that the largest mass of curds was formed in the fifth period, which coincided with the period of the largest nitrogen retention. Of the two periods, fourth and fifth, with about equal protein intake, the mass of the curds was greater when the carbohydrate intake was of a lower, and the fat intake of a higher value. Comparing further the calorific value of the diet of the fourth and fifth periods, in which the protein intake was unchanged, one notices that in the fourth period the food utilization was less perfect, and yet it led to the formation of a smaller mass of curds.

On the other hand, the stools were completely free from lumps in the second period. In that period the food intake was of a comparatively lower calorific value, and in proportion to other food-stuffs rich in carbohydrates and very poor in protein. The retention of nitrogen in that period was very insignificant. In other words, the state of nutrition was in a less perfect condition when the stools were free from curds.

CONCLUSIONS

1. The "hard" or "casein" curds represent remnants of food, principally of protein nature, that have escaped being digested.

2. The exact mechanism of their formation as yet cannot be ascertained and they should be regarded as a peculiarity appearing in course of imperfect conditions of digestion.

3. The curds are not pathognomonic of any definite pathologic condition.

4. The loss of food occasioned by their formation and the impairment of the general nutrition resulting from it is insignificant.

5. In attempting to correct the state of digestion one should be guided by the general rules of infant feeding, paying only secondary attention to the appearance or disappearance of curds from the stools.

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TABLE 5.—DANIEL L. PROTEIN RETENTION*

Number	Date	Intake in gm.			Intake in Per Cent.			Intake in Calories. Total Number Calories	Output.		Daily Average Nitrogen				Average Daily Gain in Weight	Description of Feces
		Carbohydrate, gm.	Fat, gm.	Nitrogen, gm.	Carbohydrate, Per Cent.	Fat, Per Cent.	Protein, Per Cent.		Urine, gm.	Feces, gm.	Absorbed, gm.	Retained, gm.	Absorbed, Per Cent.	Retained, Per Cent.		
	Oct. 1910															
1.	15-25	55.34	16.1	2.109	5.0	1.4	1.2	453	1.496	0.456	1.651	0.155	78.3	9.4	— 3.9	Partly pasty, smooth, gray-yellow and brown-yellow, partly watery, granular, green-brown. Hard curds present on three days.
2.	25-28	73.85	14.5	1.759	6.0	1.2	1.0	495	1.377	0.321	1.438	0.061	81.8	4.2	+12.3	Mostly formed and brown; a little thinner and green-brown. No hard curds present.
	Nov.															
3.	8-15	95.0	27.4	3.618	7.4	2.2	2.0	776	1.651	0.946	2.672	0.981	84.5	32.1	+28.7	Partly formed, smooth, brown; partly thinner, granular, yellow. Hard curds present on four days.
4.	15-22	84.04	18.0	4.831	6.3	1.0	2.55	695	3.201	0.809	4.022	0.821	83.3	20.4	+15.1	Like the feces of preceding period, generally more watery. Hard curds present every day, often large curds.
	Dec.															
5.	6-10	56.05	20.5	4.765	5.0	1.8	2.1	608	2.709	1.005	3.760	1.051	77.9	28.3	— 7.6	Mostly loose-watery (first day only formed), granular, with mucus, green-yellow, many large hard curds, some present every day.

Composition of Food

No.

1. 35 per cent. milk, 5 per cent lime-water, 60 per cent. water, milk-sugar to 5 per cent.
2. 30 per cent. milk, 5 per cent. lime-water, 65 per cent. barley water, milk-sugar to 5 per cent., 12 c.c. malt-soup extract.
3. 55 per cent. milk, 5 per cent. lime-water, 40 per cent. water, milk-sugar to 5 per cent, 30 c.c. malt-soup extract.
4. Fat-free milk 20 per cent., skimmed milk 55 per cent., lime-water 5 per cent., water 20 per cent., milk-sugar to 5 per cent., 16 c.c. malt soup extract.
5. Fat-free milk and full milk, 5 per cent. lime-water and water to make milk fat 1.8 per cent., milk protein 2.1 per cent., milk-sugar to 5 per cent.

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After the present communication was completed for publication there appeared a paper by Brennemann[7] on the etiology and nature of hard curds. On the basis of many observations the author was led to the conclusion that curds appear in stools only after feeding on raw milk, and that the curds disappear as soon as the diet is changed to boiled milk. Brennemann takes for granted that the appearance of curds is the result of a serious fault of digestion, which can be corrected by changing the diet from raw to boiled milk. The observations of Brennemann may prove of practical and theoretical importance, but his work fails to furnish new information as to the clinical significance of the appearance of the curds. The present observations may be of some value whether or not the views of Brennemann are corroborated.

Without any desire to contradict, or to disagree with the views expressed by this writer, attention may be called to the fact that the first patient under our observation was fed on boiled milk.

METHODS OF ANALYSIS

Total Nitrogen was determined by the Kjeldahl-Gunning method. Samples of food mixtures, feces, and curds were dried on a water bath and then to constant weight in an air bath.

Sugar estimation was made volumetrically by the method described by Ettore Selvatici.[8] The procedure was the following: The proteins of the milk were removed by coagulation, and the clear filtrate was added from a burette into a definite volume of a boiling Fehling's solution. A small amount of potassium ferrocyanid is added to Fehling's solution previous to the beginning of titration. The sugar solution is added until the Fehling solution loses its blue color.

Starch content in barley water, barley jelly, and bean gruel was determined by its inversion into glucose. This was accomplished by heating the solution with dilute sulphuric acid in an autoclave. The sugar was titrated by the Pavy method.

Fat estimation was made by extraction with ether in a Soxhlet apparatus. For the determination of the total fat, the substance to be extracted was carefully mixed with phosphoric acid. Fat exclusive of soap fat was estimated directly on the unmixed feces. In the food, fat was estimated by extracting with ether in a Soxhlet apparatus. In order to remove the moisture from the ether extracts, the residues of these extracts were redissolved in chloroform, filtered through paper moistened with chloroform, and dried first on a water bath and then in a desiccator.

7. A Contribution to Our Knowledge of the Etiology and Nature of Hard Curds in Infants' Stools, Am. Jour. Dis. Child., 1911, i, 341.

8. Selvatici, Ettore: Bul. de l'Assn. des Chimistes de sucrerie et de distillerie, 1910, xxvii, 1179.

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Ash-Estimation.—The dry samples of food or excretions were incinerated and the residue extracted with hydrochloric acid. The insoluble part was further ignited with the use of a few drops of strong nitric acid. The acid filtrate was added to the residue and evaporated to dryness.

The total acidity and volatile acids in the feces were determined in the following way: A known proportion of the moist feces was made into an emulsion by shaking in distilled water with glass beads, and then made to definite volume. An aliquot part was titrated for total acidity with decinormal sodic hydrate, phenolphthalein being used as indicator. To determine volatile acids, another portion of the emulsion was made acid to congo paper with dilute phosphoric acid and distilled with steam into a measured quantity of decinormal sodic hydrate until the dropping distillate was no longer acid; the unneutralized sodic hydrate then being titrated with decinormal sulphuric acid and phenolphthalein used as indicator.

HISTORIES

Daniel L., 7 months old, was admitted to the hospital Oct. 5, 1910. There was a history of protracted diarrhea and no gain in weight. He weighed 10 pounds, 8 ounces and was poorly nourished. Metabolism experiments were carried on from October 15 through October 28, from November 8 through November 22, and from December 6 through December 10, and the stools were collected for analysis until December 19. During the first period the weight was variable, being about the same at the end as at the beginning, 4,722 gm. The temperature was also variable. During the second period the child was looking well and the weight increased rather steadily from 5,003 grams to 5,310 grams. In the last period the weight was again variable, showing a slight loss at the end of the metabolism experiment and a small increase during the following days in which the stools were collected. During this time the child had low, irregular fever due to a cervical adenitis, which continued for some time after the conclusion of the experiment, accompanied by loose stools and falling weight. Subsequently the food was changed to one with less fat and protein and high carbohydrate with marked improvement and gain in weight.

Richard M., 2½ months old, was admitted to the hospital Sept. 6, 1910. He had not been gaining weight, and for ten days had had diarrhea with fever and some vomiting. He was poorly nourished but not emaciated, weighing 9 pounds, 6 ounces. He was used as a control for the Daniel L. experiments, his stools being collected from October 16 through October 28, November 8 through November 22, and December 6 through December 19. During all this time he showed a generally rising weight-curve, from 4,527 gm. on October 16 to 5,063 gm. on December 19. After the first period the stools were very good, though they frequently contained small hard curds, rarely large or numerous ones. He was discharged December 25, gaining and in good condition.

John D., 2½ months old, was admitted to the hospital Sept. 27, 1910, with a history of diarrhea and loss in weight. He was emaciated and rachitic. His weight was 5 pounds, 11 ounces. He began to gain almost at once and his stools improved. Successive increases were made in his food as his weight became stationary, on October 11, October 22, November 8, November 16, and November 23. Between November 8 and November 16 a peri-rectal abscess formed and he had fever but still continued to gain in weight. December 3 large hard curds began to appear in his stools. From December 6 through December 19 the stools were collected for analysis. During this time his stools were generally good, though they contained many large hard curds, and his weight increased from

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3,508 gm. December 6 to 3,584 gm. December 18. Double otitis media developed from December 5 and erysipelas of the scalp from about December 18, with more or less fever, and he began losing weight. He was well of the erysipelas by December 29, but one ear was still discharging at that time. The gain in weight began when he was placed on a milk formula with low fat and protein and relatively high sugar.

Joseph H., 9 months old, was admitted to the hospital Feb. 6, 1911, weight 8 pounds, 3½ ounces. There was a history of constipation and considerable colic, and recently, vomiting and loss of weight. He was rather poorly nourished. There was no vomiting after admission but his weight remained at a standstill until February 24, when his food was increased. He served as a metabolism control from March 14 through March 23. During the experiment he gained slightly in weight, from 3,955 gm. to 3,994 gm. and had good stools, which, however, invariably contained hard curds, often large and numerous ones. He continued to gain in weight and have good stools and was discharged April 23, weighing 4,583 gm.

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CHEMICAL STUDIES ON INTESTINAL INFANTILISM.

I. ENDOGENOUS METABOLISM: KREATININ, KREATIN, URIC ACID.*

BY FRANCIS H. McCRUDDEN.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

Intestinal infantilism was first recognized by the late Dr. Christian A. Herter.[1] It is a condition occurring in childhood, and is characterized by periods of disturbance of nutrition with loss of weight, alternating with periods of normal nutrition and gain of weight, and finally ending in a state of arrest of development in which it is almost impossible to bring about increase of weight. There is marked abdominal distension, a moderate grade of anemia, and a rapid onset of physical and mental fatigue. Various irregularities of digestion occur, accompanied by a reversion of the intestinal flora to the infantile type.

Dr. Herter had worked out the clinical description of the disease and begun a study of the physiological-chemical changes that accompany it, when the work was interrupted by his death. Intestinal infantilism is not a rare disease, and I have therefore continued the studies begun by Dr. Herter.

The object of the physiological-chemical studies is to determine whether there are any changes in the metabolism, and, if so, to ascertain what they are. The studies were carried out on three patients: F. S., ten years old, weight 13.5 kilograms, and height 96 centimeters; J. P., seventeen years old, weight 22.5 kilograms, and height 113.3 centimeters; and F. H., five and a half years old, weight 12.0 kilograms, and height 91 centimeters. All three patients have histories of typical intestinal infantilism. F. S. and J. P. had

* Received for publication, December 4, 1911.

[1] Herter, Christian A., On Infantilism from Chronic Intestinal Infection, New York, 1908.

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been under observation by Dr. Herter for a long time, and F. H. had been under the care of Dr. L. Emmett Holt. At the time that they came to the Rockefeller Institute Hospital they had reached the stage of arrest of development, although there were occasional periods of slight improvement, which I shall mention later.

The present paper reports the study of the metabolism of uric acid, kreatinin, and kreatin, which was undertaken to throw light on the endogenous metabolism of the cases. It is now recognized that when meat, fish, and a very few other foods are excluded from the diet, the uric acid and kreatinin of the urine come entirely from the metabolism of the tissues themselves, and that kreatin, under these conditions, does not normally occur in the urine; so that the quantities of these substances arising on a meat-free diet serve as a measure of the endogenous metabolism,—the metabolism of the tissues as distinct from the direct metabolism of the substances in the food.

In the cases of F. S. and J. P., examinations were made during three separate periods. During the second and third periods, the patients were in much better condition than in the first period; and the examinations of the later periods were made with the object of finding out what changes such variations in the condition of the patient make in the results. The diet in all the experiments was free from foodstuffs that are known to give rise to purin bodies, kreatinin, and kreatin, and consisted chiefly of milk, eggs, bread, and rice in quantities that satisfied the appetite. The results are given in table I.

The endogenous uric acid should be fairly constant for one individual, and not variable, as in these cases. It is not possible to explain the irregularities, since we do not know all the factors that determine the amount of endogenous uric acid, which varies in different individuals. It does not appear to be related to the general metabolism, as represented by the nitrogen excretion, or to the weight or age of the individual.

It will be noticed that the kreatinin varies in the different periods, especially in the case of F. S. This is due in part to a gradual increase in body weight. At the same time, the daily variations, especially in J. P., are somewhat greater than one would expect

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from the results that I have obtained in normal persons. There are not enough data from normal individuals available for comparison to determine whether the absolute amount of kreatinin per kilo of body weight—the kreatinin coefficient—is normal in these cases. The normal amount for an adult is between 0.02 and 0.03 of a gram per kilo of body weight. In infants, the kreatinin coefficient is much smaller;[2] and, indeed in young children, kreatinin is sometimes absent.[3] I have found in the literature no data for half-grown children. In one boy of thirteen, of about the same weight

TABLE I.[4]

	Date.	Nitrogen.	Uric acid.	Kreatinin.	Body weight in kilos.	Kreatinin per kilo of body weight.	Kreatin.	Total of kreatinin + kreatin
F. S.								
1st period	Dec. 21, 1910	3.02	0.314	0.150	14.0		0.004	0.154
	Dec. 22, 1910	2.75	0.321	0.155		0.011	0.023	0.178
	Dec. 23, 1910	2.51	0.285	0.175			0.012	0.187
	Dec. 24, 1910	2.32	0.252	0.169			0.000	0.169
2d period	March 3, 1911	4.31	0.396	0.194	16.5	0.011	0.055	0.249
	March 4, 1911	—	0.343	0.171			0.052	0.223
3d period	March 21, 1911	4.17	0.292	0.213	17.0		0.091	0.304
	March 22, 1911	3.56	0.392	0.198		0.014	0.055	0.253
	March 23, 1911	3.97	0.448	0.203			0.059	0.262
J. P.								
1st period	Dec. 20, 1910	7.72	0.122	0.307	21.0		0.047	0.354
	Dec. 21, 1910	5.53	0.130	0.263		0.013	0.014	0.277
	Dec. 22, 1910	5.55	0.145	0.248			0.032	0.280
	Dec. 23, 1910	5.91	0.184	0.297			0.051	0.348
2d period	March 3, 1911	3.52	0.130	0.284	21.0	0.014	0.020	0.304
	March 4, 1911	—	0.141	0.289			0.015	0.304
3d period	March 21, 1911	5.86	0.229	0.346	21.5		0.071	0.417
	March 22, 1911	4.64	0.151	0.312		0.015	0.040	0.352
	March 23, 1911	4.95	0.150	0.317			0.045	0.362
F. H.	March 10, 1911	5.68	0.172	0.169	12.0		0.094	0.263
	March 11, 1911	5.44	0.126	0.169			0.081	0.250
	March 12, 1911	5.27	0.087	0.169		0.014	0.087	0.256
	March 13, 1911	5.62	0.159	0.158			0.116	0.274
	March 14, 1911	5.87	0.132	0.173			0.112	0.285
	March 15, 1911	5.56	0.064	0.181			0.135	0.316

[2] Amberg and Morrill, *Jour. Biol. Chem.*, 1907, iii, 311.

[3] Hoogenhuyze and Verploegh, *Ztschr. f. physiol. Chem.*, 1905, xlvi, 415.

[4] Kjeldahl's method was used for nitrogen determinations, and Folin's methods for the other determinations.

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as J. P., I found the coefficient to be that of the adult, so that the kreatinin in the cases of infantilism is probably very low. It may be mentioned in this connection that in starvation Cathcart[5] found a slight gradual lowering of the kreatinin excretion.

Kreatin was always present in the urine of my patients, even on a meat-free diet, and the presence of kreatin in human urine is considered abnormal.[6] It is found in different pathological conditions, especially in those associated with disturbance of the metabolism of the muscular system or of the liver, or in conditions in which the carbohydrate metabolism is disturbed. The cause of the presence of kreatin in the urine in these cases is not known, but it can be caused to disappear by the administration of carbohydrates, but not by fats.[7] These experiments led me to try the effect of a carbohydrate and fat diet on the kreatin excretion in my patients.

The amounts of kreatin and kreatinin were determined during three periods; a preliminary period, a period during which much fat was given, and a period when a large quantity of carbohydrates was given. In all three periods, the diet was kreatin-free, and was made up chiefly of rice, milk, toast, crackers, and fruit. During the second period, the carbohydrates were cut down as much as possible, and cream and butter were given in as large quantities as the patient could be induced to take. The amounts varied on different days; but, on the average, the amount given was about seventy grams of cream and ninety grams of butter. During the third period, fats were cut down as much as possible, and rice, bread, potatoes, cake, sugar, and starch puddings were given in as large quantities as possible. The results are shown in table II.

It will be observed that, in contrast to the results obtained in the experiments of the investigators who studied the effect of carbohydrate and fat diet on kreatin excretion in cases where there was disturbance of the carbohydrate metabolism, the fat diet, in the

[5] Cathcart, *Biochem. Ztschr.*, 1907, vi, 109.

[6] Rose (*Jour. Biol. Chem.*, 1911, x, 265) found kreatin in the urine of children on a mixed diet, but there is the possibility of tracing this to the kreatin in the food. Kreatin in the food may appear under some circumstances in the urine of normal persons (Folin, Festschrift, Olof Hammarsten, Upsala, 1906).

[7] In man, during inanition, Cathcart, *Jour. Physiol.*, 1909, xxxix, 311; in dogs, during phlorizin diabetes, Cathcart and Taylor, *Jour. Physiol.*, 1910–11, xli, 276; in starving rabbits, Mendel and Rose, *Jour. Biol. Chem.*, 1911, x, 213.

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cases of infantilism, led to a lower kreatin output than the carbohydrate diet. The larger figure in the first days of the preliminary period may be due to a washing out of kreatin from the food taken before the experiment began, but there are not sufficient data regarding the factors that influence kreatin excretion to explain the results.

TABLE II.

	F. S.					J. P.			
	Kreatinin.	Kreatin.	Average.	Total.		Kreatinin.	Kreatin.	Average.	Total.
May 22, 1911	0.196	0.137		0.333	Preliminary period	0.307	0.093		0.400
May 23, 1911	0.183	0.137	0.121	0.319		0.441	0.109	0.073	0.550
May 24, 1911	0.127	0.098		0.225		0.362	0.017		0.379
May 25, 1911	0.265	0.073		0.338		0.324	0.034		0.358
May 26, 1911	0.203	0.028	0.050	0.230	Fat-rich diet	0.273	0.029	0.030	0.302
May 27, 1911	0.166	0.059		0.225		0.405	0.026		0.431
May 28, 1911	0.225	0.143		0.368	Carbohydrate-rich diet	0.335	0.054		0.389
May 29, 1911	0.191	0.099	0.076	0.290		0.353	0.015	0.038	0.368
May 30, 1911	0.205	0.086		0.290		0.324	0.044		0.368

There is a rather low sugar tolerance in my cases, and this may have some bearing on the findings relating to kreatin. On April 20th, at 3:00 P. M., both boys were given fifty grams of glucose. The urine collected in the next hour showed a strong reduction in the case of J. P., but no reduction in the case of F. S. The next day F. S. was given seventy-five grams of glucose and J. P. 25 grams, and the urine of the following hour showed a faint reduction in both cases. F. S. showed a glucose tolerance, under these circumstances, of between fifty to seventy-five grams, and J. P. one of between twenty-five and fifty grams. A similar experiment to test levulose tolerance was made, but the patients found it difficult to eat more than twenty-five grams. The urine of the following hour showed, on being boiled with Fehling's solution, a doubtful reduction.

The following explanation for the presence of kreatin and acetone bodies in the urine in the diabetic condition and in starvation, has been suggested, and may have some bearing on the cases of infantilism. In diabetes and starvation, the body utilizes its own protein material to form carbohydrates, since direct supplies are

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cut off, and the excessive metabolism of muscular tissue is accompanied by the production of kreatin and acetone bodies. If carbohydrates are administered, muscle protein is protected and the appearance of kreatin and acetone bodies, which accompanies the excessive metabolism of tissue protein, is prevented.[8] In intestinal infantilism, it may be that fat is utilized to better advantage than carbohydrates, and, therefore, better protects the increased tissue catabolism which may be responsible for the presence of kreatin.

SUMMARY.

In my three cases of intestinal infantilism, the kreatinin excretion was more irregular than normally, and the kreatinin coefficient was low. Kreatin was found in the urine on a kreatin-free diet. The sum of kreatinin and kreatin was also irregular.

The endogenous uric acid was irregular from day to day.

The kreatin excretion is apparently lower on a high fat diet than on a high carbohydrate diet.

[8] Fatta, Grote, and Staehelin, *Beitr. z. chem. Phys. u. Path.*, 1907, x, 199.

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CHEMICAL STUDIES ON INTESTINAL INFANTILISM.

II. THE RELATION OF ENDOGENOUS TO EXOGENOUS METABOLISM: NITROGEN AND SULPHUR DISTRIBUTION, AND CALORIMETER EXPERIMENTS.*

BY F. H. McCRUDDEN AND H. L. FALES.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

The studies reported in this paper were carried out on the same three patients as those described in a previous paper,[1] and on a normal boy for comparison. The normal boy, W. McC., was eleven years old and weighed twenty-seven kilos.

The presence of kreatin, and, at one time, of acetone, in the urine of the abnormal cases, and of acetone in the breath of J. P., and the very large amounts of various elements lost through the feces of these patients, made it seem possible that the lack of absorption was leading to some condition of starvation that was responsible for the arrest of development.

The first part of the paper deals with the experiments relating to the distribution of nitrogen and sulphur in the various nitrogen and sulphur compounds in the urine, which were carried out in order to test the starvation supposition. In normal nutrition, over 80 per cent. of the nitrogen is excreted in the form of urea, and about 80 per cent. of the total sulphur in the form of inorganic sulphates. The fraction of nitrogen and sulphur occurring in other forms is very low. In starvation or in a low protein diet, the proportions of total nitrogen as urea, and of total sulphur as sulphates, that indicate the degree of exogenous metabolism, are low, and the sulphur and nitrogen in forms that indicate more especially the degree of endogenous metabolism are high. A study

* Received for publication, December 4, 1911.

[1] McCrudden, *Jour. Exper. Med.*, 1911, xv, 107.

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TABLE I.[a]

	Nitrogen.							Sulphur.					
	Total.	Urea—N.		Ammonia—N.		Amino—N.		Total.	Inorganic.		Ethereal.		Neutral.	
		Absolute amount.	Per cent. of total.	Absolute amount.	Per cent. of total.	Absolute amount.	Per cent. of total.		Absolute amo t.	Per cent. of total.	Absolute amount.	Per cent. of total.	Absolute amount.	Per cent. of total.
F. S. (infantilism)														
Weight 16.4 kilos.														
Feb. 15, 1911	5.57	4.74	85.2	0.37	6.5	0.38	6.8	0.391	0.304	77.8	0.052	13.3	0.035	9.0
Feb. 16, 1911	5.12	4.16	81.4	0.22	4.3	0.42	8.2	0.320	0.224	70.0	0.040	12.5	0.056	17.5
Feb. 17, 1911	4.48	3.58	80.0	0.24	5.4	0.31	6.9	0.288	0.224	77.8	0.024	8.3	0.040	13.9
Feb. 18, 1911	5.12	4.32	84.4	0.10	2.3	0.30	5.9	0.344	0.276	80.3	0.036	10.5	0.032	9.3
J. P. (infantilism)														
Weight 21.3 kilos.														
Feb. 15, 1911	5.48	4.53	82.8	0.19	3.5	—	—	0.396	0.325	82.2	0.029	7.3	0.042	10.6
Feb. 16, 1911	5.35	4.75	87.8	0.12	2.2	0.13	2.4	0.353	0.315	89.4	0.010	2.8	0.028	7.9
Feb. 17, 1911	5.04	4.16	82.6	0.15	3.0	0.26	5.2	0.324	0.276	85.2	0.026	8.0	0.022	6.8
Feb. 18, 1911	4.86	4.12	84.7	0.06	1.5	0.31	6.4	0.286	0.250	87.4	0.020	7.0	0.016	5.6
F. H. (infantilism)														
Weight 12.0 kilos.														
March 10, 1911	5.68	4.96	87.4	0.264	4.7	—	—	0.432	0.378	87.6	0.018	4.2	0.036	8.4
March 11, 1911	5.44	4.80	88.2	0.228	4.2	—	—	0.412	0.362	87.9	0.022	5.4	0.028	6.8
March 12, 1911	5.27	4.64	88.0	0.260	4.9	—	—	0.392	0.334	88.3	0.022	5.6	0.036	9.2
March 13, 1911	5.62	4.94	88.1	0.256	4.6	—	—	0.428	0.368	86.2	0.028	6.5	0.032	7.5
March 14, 1911	5.87	5.10	87.0	0.212	3.6	—	—	0.452	0.390	86.5	0.022	4.9	0.040	8.9
March 15, 1911	5.56	4.94	88.8	0.152	2.7	—	—	0.424	0.368	86.9	0.020	4.7	0.036	8.5
W. McC. (normal boy)														
Weight 27.1 kilos.														
April 8, 1911	10.43	9.86	94.5	0.608	5.8	—	—	0.729	0.632	86.7	0.008	1.1	0.089	12.2
April 9, 1911	10.65	8.93	83.8	0.498	4.7	—	—	0.744	0.648	87.2	0.000	0.0	0.096	12.9
April 10, 1911	10.35	8.71	84.2	0.404	3.8	—	—	0.717	0.652	90.8	0.010	1.4	0.055	7.7
April 11, 1911	11.07	9.20	83.2	0.408	3.7	—	—	0.832	0.724	87.2	0.024	2.9	0.084	10.1
April 12, 1911	11.35	9.79	86.2	0.441	3.9	—	—	0.808	0.718	87.8	0.017	2.1	0.073	9.0
April 13, 1911	11.12	9.25	83.2	0.510	4.6	—	—	0.810	0.696	85.9	0.017	2.1	0.097	12.0

[a] Methods: for nitrogen, Kjeldahl; for ammonia, Folin (*Ztschr. f. physiol. Chem.*, 1902, xxxvii, 161); for amino acid

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of the nitrogen and sulphur distribution in an individual case shows which of these two states of nutrition probably prevails.

The determinations were made also for the purpose of studying the bacterial conditions in the intestines, as revealed by the changes in the ethereal sulphates. The attack of acetonuria referred to led us to investigate the ammonia excretion, as being indicative of the degree of acidosis.

The second part of the paper deals with the determination of the respiratory quotient after eighteen hours' starvation, and gives certain data regarding the heat radiation. The under-development of the individual, the presence of kreatin, and, at one time, of acetone in the urine, suggested that there might be some difficulty in elaborating carbohydrates, and that, as a consequence, the store of glycogen might be low. If this were so, a low respiratory quotient, indicating the oxidation of fat, might be expected after eighteen hours' starvation.

NITROGEN AND SULPHUR DISTRIBUTION.

The diet was a mixed one, and the patients were allowed to eat about what they wanted. Table I gives the data.

It will be seen that the fractions of nitrogen excreted as urea, and of sulphur excreted as inorganic sulphates, are 80 per cent. and over.[8] These are normal figures and indicate the usual ratio between endogenous and exogenous metabolism. The children appeared, therefore, to be absorbing a sufficient quantity of protein for their metabolism.

TABLE II.

	Average nitrogen excretion in grams per day.	Body weight in kilos.	Body surface[4] in square meters.	Nitrogen in grams per square meter of body surface.
F. S. (infantilism)	5.07	16.4	0.794	6.38
J. P. (infantilism)	5.18	21.3	0.946	5.48
F. H. (infantilism)	5.57	12.0	0.645	8.60
W. McC. (normal boy)	10.83	27.1	1.190	9.10

[8] In F. S., the percentage of inorganic sulphur is slightly lower on account of the large quantities of ethereal sulphates.

[4] Calculated by the formula, body surface $= 12.3 \sqrt[3]{(\text{body weight})^2}$. Lusk, The Elements of the Science of Nutrition, 2d edition, Philadelphia and London, 1909, 89.

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That the metabolism is on a low plane may be seen from a calculation of the amount of nitrogen metabolized per square meter of body surface (table II).

The excretion of nitrogen through the urine in cases of infantilism is much less than in the normal individual, and, except in the very mild case of the boy F. H., is less than the value (6.4 in man)[5] in starvation. This is not because an insufficient amount of protein is given in the diet, for, aside from the fact that the patients were given as much as they could well take, the nitrogen and sulphur distribution in the urine indicates that, for these individuals, the amount was sufficient. In connection with this evidence of low metabolism, the low kreatinin fraction found in a previous experiment should be remembered.[6]

The absolute amount of ammonia excreted is low and shows that there is no tendency toward acidosis. The absorption of salts is sufficient to neutralize the acid end products of metabolism, and the carbohydrate metabolism is sufficiently active to prevent any abnormal oxidation of the fats.

The absolute and the relative quantities of ethereal sulphates are slightly, but not excessively high. This probably indicates a slightly increased, but not excessive amount of intestinal putrefaction, although not more than can be accounted for by the fact, observed in other experiments, that absorption from the intestines is poor.

CALORIMETER EXPERIMENTS.[7]

The apparatus and methods used in these experiments are described briefly by Howland[8] and will be more completely described by Williams.[9] Our results are given in table III. The first column gives the outgo of carbon dioxid, the second column shows the intake of oxygen, and the last column gives the respiratory coefficient.

[5] Voit, E., *Ztschr. f. Biol.*, 1901, xli, 167.

[6] McCrudden, *loc. cit.*

[7] Dr. Graham Lusk, of Cornell University Medical School, kindly put his calorimeter apparatus at our disposal for these experiments, and Dr. H. B. Williams and Mr. J. A. Riche, of the Department of Physiology, conducted the experiments.

[8] Howland, J., *Ztschr. f. physiol. Chem.*, 1911, lxxiv, 1.

[9] Williams, H. B., cited by Howland, *loc. cit.*

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The patient had received no food since 5:00 P. M. on the preceding day.

TABLE III.

	Carbon dioxid.	Oxygen.	Respiratory coefficient.
11.10 A.M. to 12.10 P.M.	10.00	9.52	0.76
12.10 P.M. to 1.10 P.M.	9.90	8.97	0.82
Average			0.79

The respiratory quotient indicates oxidation of a mixture of fat and carbohydrates, and shows that the glycogen store has not been used up during eighteen hours' starvation.

Table IV gives examples of the heat radiation, which is about normal.

TABLE IV.

	Heat found.	Heat calculated.	Calories per square meter of surface.
1.34 P.M. to 2.34 P.M.	36.68	37.38	929 (awake)
2.34 P.M. to 3.34 P.M.	32.72	32.81	840 (asleep)

DISCUSSION.

Our conclusions, then, are as follows: (1) that the metabolism, as a whole, is on a low plane, as is indicated by the fact that the nitrogen excretion per square meter of body surface is at about the starvation value; (2) that the endogenous metabolism is low, as is shown by a low kreatinin coefficient;[10] (3) that the distribution of nitrogen and sulphur among the different compounds in the urine is in the ratio found in normal persons only when the metabolism is on a high plane.

From the data that we have obtained, we feel justified in suggesting certain possibilities, the value of which can only be proved by further investigation. It may be suggested that possibly some fundamental defect exists that prevents the body from engaging in a more active metabolism. Or there may be a long continued state of under-nutrition due to poor absorption,[11] which, at first, leads only to a low exogenous metabolism, but in time is followed

[10] McCrudden, *loc. cit.*

[11] Later studies have shown that absorption is very poor.

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by a readjustment whereby the endogenous metabolism gradually falls to a lower plane, and finally exogenous and endogenous metabolism come to the normal balance in such a way that nitrogen and sulphur distribution again become normal. Only one instance of previous study of the nitrogen and sulphur distribution after a very long period of low nitrogen diet has come to our attention.[12] In that experiment, it is true, the body did not adjust itself in the way we have suggested, but it may be remarked that the conditions may have been different. However, that the endogenous metabolism may show a gradual decrease in starvation is indicated by the gradual lowering of the kreatinin excretion.[13] A final possibility is that one or more of the amino acid complexes of the food protein are so changed by bacterial action in the intestines that they cannot be utilized by the body.

SUMMARY.

In three cases of intestinal infantilism, the excretion of nitrogen in the urine per square meter of body surface was low.

The heat radiation was normal.

In spite of the fact that the absolute quantity of nitrogen was low, the distribution of nitrogen and sulphur among the various nitrogen and sulphur constituents of the urine was in the normal ratio.

The ammonia excretion was normal.

The ethereal sulphates were slightly increased.

The respiratory coefficient showed that the supply of glycogen had not been used up after eighteen hours' starvation.

[12] Folin, *Am. Jour. Physiol.*, 1905, xiii, 66.
[13] Cathcart, *Biochem. Ztschr.*, 1907, vi, 109.

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THE BEHAVIOR OF CHICKEN SARCOMA IMPLANTED IN THE DEVELOPING EMBRYO.*

BY JAMES B. MURPHY, M.D., AND PEYTON ROUS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 17–22.

Current theories of tumor origin have done much to stimulate the experimental comparison of neoplastic with embryonic tissue. The discussions upon "cell rests," "cell avidity," and the mutual relationships of tissues as determining the incidence of tumors (Thiersch), have led to a vast amount of work in this field. But the methods used have, of necessity, been indirect. In the present paper we wish to offer some observations on the direct transmission of tumors to the developing embryo.[1]

The common transmissible tumors have occurred in mammals, and it is most difficult to inoculate the growth into the mammalian embryo *in utero* without interrupting the course of pregnancy. Nevertheless it can be done, as one of us (Rous) has found. With a fine, hollow needle, injections of tumor pulp were made through the uterine wall into rat embryos; and some of these went on to term. In one instance, the tumor graft was found in an animal killed just after birth, but in the few days previous to the end of pregnancy, it had only established itself and had not grown. Embryos inoculated early in gestation did not survive.

The problem is much simpler when use is made of developing hen's eggs and the avian sarcoma propagated in this laboratory.[2] Under suitable precautions, the injection into the chick or its membranes need do no more than transiently retard development. We have employed the method developed by Peebles[3] for studies in

* Received for publication, December 12, 1911.

[1] Peyton Rous and James B. Murphy, *Jour. Am. Med. Assn.*, 1911, lvi, 741.

[2] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

[3] F. Peebles, *Arch. f. Entwcklngsmechn. d. Organ.*, 1898, vii, 405.

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experimental embryology. A small rectangular piece is cut from the shell with a sharp instrument, care being taken not to break the underlying membranes; with fine, sterile forceps, the shell membrane is torn and pulled back, exposing the chick and its membranes, and through a hollow needle the tumor material is placed in the desired location. The bit of shell is then put back and sealed into position with paraffin, or with narrow strips of moist shell membrane from a fresh egg. For the purpose of inoculation, the tumor is reduced to a pulp, either by grinding or by forcing it through a sieve; and a very minute portion is injected into each egg. To regulate the amount actually implanted in the tissue is very difficult, as that which it is intended to place in an extra-embryonic membrane largely escapes to either side, and that implanted in the embryo itself often follows the needle to the surface. The eggs of pure blooded, barred, Plymouth Rock chickens have been mainly used, both for purposes of comparison, and because adult fowls of this variety are especially susceptible as hosts for the sarcoma.

The results include observations upon 147 hen's eggs, 16 duck, and 9 pigeon eggs, which continued to develop after inoculation.

GENERAL DESCRIPTION OF THE FINDINGS.

In 108 of the 147 hen's eggs, the inoculation gave rise to one or more tumors. The findings varied with the stage at which the embryo was inoculated, and were further complicated by its development. For a preliminary general description use will be made of the growths in eggs inoculated on the seventh or eighth day of incubation, and opened a week or ten days later. The best success was obtained under these conditions.

Tumors in the Outer Allantoid Membrane.—The majority of the growths occurred in the outer allantoid membrane (fused chorion and allantois), and for evident reasons. This membrane was necessarily pierced in reaching other structures, and its inoculation could hardly be avoided in withdrawing the needle. Often the material expelled elsewhere from the needle clung, and was dislodged only on the inner surface of the allantoid membrane.

When the egg successfully inoculated is opened on the fifteenth

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or sixteenth day of incubation, a disc-shaped, gray opacity is seen at the point where the allantoid membrane was wounded by the needle. It is sharply defined, but not raised, and suggests scar tissue only. Its center may be perforated where healing has been incomplete. When the membrane is cut and turned back, a remarkable picture presents itself. From the inner surface, a discrete growth projects, glistening, smooth, and grayish pink, firm, opaque, more or less globular in shape, and sessile on the membrane (figures 1, 2, and 7). Should the membrane have failed to heal together, the growth takes the form of a sharply raised ring about the hiatus. Numerous dilated vessels course to and over the tumor. The contrast between the filmy membrane and the bulky mass which it supports is very striking.

The main growth is always situated at the point injured by the needle; but one or several, small but similar nodules are sometimes present near by, along the vessels. They also are discrete and project sharply. As early as four days after the inoculation, the implanted tissue may be vascularized and established; while after ten days a mass 1.9 centimeters in diameter may have developed. In consistence, it is softer than a nodule of the same size in the adult fowl. Necrosis is uncommon; hemorrhage into the tumor substance, less so (figure 2).

Tumors in the Other Membranes.—When at the seventh day of incubation, the injection needle is thrust toward the egg's center, it may either (*a*) penetrate the fused inner allantoid and amniotic membranes, with the amniotic sac and embryo, or (*b*) pass through the inner allantoid membrane into the extra-embryonic body cavity and yolk-sac (figure 3). When the inoculation has taken place according to the first possibility, a small tumor is usually found at the point where the needle has pierced the fused amnion and allantoid membrane. In general appearance, the growth resembles those already described, but it is usually much smaller, lies in the plane of the membrane and projects sharply on either surface. Its vascularization is less profuse than in the outer allantoid membrane, which probably in part explains its small size.

When the inoculation has occurred according to the second possibility, namely, through the inner allantoid membrane into the extra-

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embryonic body cavity and yolk-sac, a most interesting variation is encountered. The tumors heretofore discussed have been localized at the point wounded by the injection needle; but when the material has been introduced into the extra-embryonic body cavity, many scattered growths appear on the surface of the inner allantoid membrane, the amnion, and the yolk-sac, which together enclose it. Their lining surfaces, in distinction from those of the membranes heretofore dealt with, consist of mesoderm (figure 3). The growths that form upon this mesodermal layer are discrete, pearly, globular or disc-shaped, and of various sizes, though usually small; and they lie, not within the substance of the membrane, but upon its surface to which their attachment may be slight (figure 4). In the yolk-sac, there frequently occurs at the point punctured by the needle, a large tumor, discrete as in the other membranes, grayish pink, and translucent. It is raised only slightly on the outer surface of the sac, but may develop considerably towards its interior, forming a conical mass projecting inwards.

Tumors in the Embryo.—Thus far no attempt has been made to implant the sarcoma in special regions of the embryo. The latter swings about under pressure with the needle, rendering this difficult. By indiscriminate implantation, growths have been obtained in the chest wall, heart, liver, peritoneal surface of the gizzard, soft tissue of the thigh and that of various other parts (figures 5 and 6). All appeared in the track of the needle. The sarcomatous nodules were similar in consistency to those in the membrane, often absolutely discrete, and always singularly non-invasive, when compared with those in the adult. Some reached a considerable size, relative to the bulk of the embryo. In several cases, we have thought that death of the embryo was to be accounted for by the presence of large tumors in the membranes. Maceration quickly ruins the material for histological purposes.

HISTOLOGICAL FINDINGS.

Histologically, the tumors differ little from those in the adult fowl. In general structure, they are quite unlike the scar tissue produced in the embryonic tissues by a trauma, or the proliferative reaction that follows the injection of infusorial earth, although

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the sarcoma cells have a resemblance to certain individual elements present in both these cases. With infusorial earth, it is possible to obtain pseudo-tumors in the outer allantoid membrane, consisting of many folds of the latter with an edematous, cellular mesoderm, covered by somewhat thickened ecto- and endoderm. All gradations are seen, here and in scar tissue, between the ordinary, stellate, mesodermal cells and spindle-shaped elements irregularly grouped. There are, in addition, polymorphonuclear and large mononuclear cells attesting the inflammatory nature of the growth. In the tumor, on the other hand, the cells are all of one type—spindle cells in bundles—while the general aspect of the neoplasm is entirely homogeneous. Within it there is absolutely no focal arrangement, and about it no cellular reaction (figure 8).

In the embryo, the structure of the sarcoma is much looser than in the adult, and the spindles may be so attenuated as to suggest that the growth is a myoma (figure 9), a possibility ruled out by the use of special stains. Intercellular fibrils are demonstrable, but are few in number. The many blood-vessels are thin walled, often consisting of only a layer of endoderm, and, apart from them, the growth possesses practically no supporting tissues. Well defined lymph-channels are not seen. The rarity of necrosis is doubtless due to the loose structure and the excellent vascularization, both of which, on the other hand, favor hemorrhage (figure 2). In general, the sarcomatous cells are larger than the normal connective-tissue elements, and their cytoplasm stains more deeply with methylene blue. The nucleus is large, oval or elongated, vesicular, with a fine net-work of chromatin and a well defined, deeply staining nucleolus. Proliferation takes place mostly by amitosis. As many as 69 per cent. of the cells, by count, have been found in process of division at one time (table I).

The growth of tumors situated in the membranes is almost wholly expansive in character. Invasion of the neighboring structures is seldom well marked. The soft, normal tissues are pressed to one side, and the mass retains microscopically the well defined border seen in the gross. So too in the embryo, except when the growth lies in structures (heart and liver) which may be supposed to adjust themselves less easily to it, and which are in consequence

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infiltrated, as well as thrust to one side. The important influence of mechanical factors on the relations of the growth is well illustrated by the findings in the outer allantoid membrane. Taking the course of least resistance, the neoplasm enlarges in the direction of the allantoid cavity and finally comes to lie almost wholly without the membrane that supports it, although still covered by endoderm and to be considered as having place in the mesoderm.

RELATION OF THE SARCOMA TO THE MESODERM.

It has been shown that in adult fowls the cells of the chicken sarcoma survive transplantation, and, proliferating, give rise to the new nodule. The process has not been followed systematically in the embryo, although it is very evident there, being perhaps best seen in the survival of sarcomatous fragments which have lodged on the exposed mesoderm lining the extra-embryonic body cavity (figure 10). These surface implantations in time become vascularized and eventually develop into neoplastic masses of considerable size.

In the adult fowl, it is not always necessary for the development of tumor fragments that they be brought directly in contact with connective tissue. Bits injected into the peritoneal cavity may establish themselves despite its endothelial lining, proliferating sometimes in a way that suggests cell cultures in a fluid medium, and at length obtaining from the underlying tissues a vascularization. Not so in the extra-embryonic membranes of the chick, according to our experience. Here a layer of ectoderm or endoderm but one cell thick constitutes an absolute protection against surface implantations. Only where a mesodermal layer is exposed, as on the inner side of the inner allantoid membrane, do such implantations occur. This dependence of the tumor cells on a direct association with mesodermal tissue, if they are to become established in the embryo, is responsible for the difference in localization of the tumors in the different membranes—for the occurrence in one of a single nodule at the puncture point, and in another of many surface growths. Recognizing this dependence and knowing the state of development of the egg, and the structures pierced by the injection needle, we can predict with some accuracy, despite the changing conditions, the localization of growths in the membranes.

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GROWTH OF SARCOMA CELLS IN THE YOLK.

The presence of a free "rice-body," consisting of tumor cells, in the extra-embryonic body cavity of an inoculated chick, has led to some experiments after the method of Burrows[4] on the *in vitro* growth of sarcoma cells in the fluids of the egg. The "rice-body" in question was rounded, gray, translucent, firm, five millimeters in diameter, was unprovided with blood-vessels or with an epithelial or endothelial covering, and was made up entirely of neoplastic cells of highly attenuated form (figure 11). To judge from the uniform failure of our attempts to grow sarcoma cells *in vitro* in the fluid of the extra-embryonic body cavity, the "rice-body" must at one time have been attached to the membrane and have derived its nourishment therefrom. The cells will not grow in egg albumen taken at various periods in the chick's development. In the yolk, on the other hand, they sometimes proliferate *in vitro*, a fact not very significant, considering the relatively simple fluids in which the proliferation of normal cells can take place.[5]

EFFECT OF THE EMBRYO'S PERIOD OF DEVELOPMENT.

Most of the embryos inoculated at very early stages succumbed to the trauma, and of those which survived, few showed the growth. This is perhaps traceable to a change in the method of inoculation, made necessary by the conditions. Injections into the blastoderm could hardly be practised; so, instead, a minute bit of tumor was placed on the tip of a fine needle and this was thrust into the desired location. Twenty-three eggs in all, which had been incubated for twenty-four to forty-eight hours, continued to develop after an inoculation of this sort into the outer zone of the blastoderm. In five, the tumor developed as a discrete, pearly nodule imbedded in the membrane, usually minute and always very small as compared with growths obtained after the same time in the membranes of older chicks. It was always found close to the margin of the area vasculosa, a fact that indicates that the proliferation of the blastoderm to form the membrane took place to a much greater degree in

[4] M. T. Burrows, *Jour. Am. Med. Assn.*, 1910, lv, 2057.

[5] M. R. Lewis and W. H. Lewis, *Bull. Johns Hopkins Hosp.*, 1911, xxii, 126.

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the inner zone than in the tissue marginal to the inoculated spot. With embryos inoculated at somewhat later periods, similar results were obtained.

When incubation was far advanced at the time of inoculation, and the chick was allowed to hatch, the tumors that had developed in the outer membranes were cast off with them. Growths in the yolk sac were drawn with it into the body and continued to develop. In one instance, a chick lived for three weeks after hatching. On autopsy, multiple sarcomatous nodules were discovered in the lungs and liver. They were best developed in the latter organ, and were presumably secondary to a tumor in the yolk-sac, now absorbed.

METASTASES.

Regional metastases in the outer allantoid membrane are fairly common. They take the form of small nodules near the primary growth, along the large vessels supplying the latter, and are presumably distributed by the lymphatics. Remote metastases from growths in the membranes have not been found, a fact which can scarcely be explained by the short period of observation, since this period suffices for the development of regional metastases. It is much better accounted for by the structure of the membranes. The lack of invasive extension in them has already been commented upon. Growth into the blood-vessels of a membrane is of rare occurrence, and even when present need not involve, in a region so protected against trauma, the casting off of cells into the blood-current.

In the embryo itself there has been no case in which the situation of the tumors could not be explained by direct inoculation with the injection needle; but our data are hardly sufficient to permit any conclusion as regards the frequency of secondary growths. In the chick that has hatched, a distribution of the sarcoma may take place by the blood-stream, as in the case of adult fowls.

TRANSPLANTATION FROM EMBRYO TO EMBRYO.

The transplantation of the sarcoma from embryo to embryo is not difficult, but in practice is limited by the small amount of ma-

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terial available for the inoculation and the high incidental mortality. By using the outer allantoid membrane as the injection site, and employing the growths found there after seven days, we have successfully transplanted the sarcoma to three successive sets of eggs, only to lose it on the fourth by death of all the embryos. The same cause terminated more quickly two other series. During the continued propagation, no change was noted in the tumor's behavior. Inoculation from the embryo into the adult yields the characteristic sarcoma.

TUMORS PRODUCED BY A BERKEFELD FILTRATE.

The chicken sarcoma has been repeatedly engendered in adults by means of the Berkefeld filtrate of an extract of the fresh tumor in Ringer's solution;[6] and so, too, in the embryo. One of us is now engaged in a study of the histological processes involved. In the egg, the growths are first evident three or four days after the injection, as minute, whitish dots, having the same location in the various structures as those produced by the living cells. For example, in the outer allantoid membrane and others with a protected mesoderm, a single growth appears at the point punctured by the injection needle; but on the exposed mesodermal lining of the extra-embryonic body cavity, widely scattered growths develop. But even on the exposed mesoderm, the neoplastic change is punctate and the nodules remain discrete, from which it may perhaps be inferred that the agent giving rise to them is not in solution but is a formed body.

The sarcoma has also been produced in the embryo by injecting a suspension of the dried and powdered neoplastic tissue. The results do not differ essentially from those obtained with a filtrate.

TUMORS IN THE PIGEON AND DUCK EMBRYOS.

The sarcoma will not grow in pigeons or ducks, while in chickens of another variety than that in which it originally occurred, it grows relatively slowly and is subject to retrogression. But in the embryos of these alien fowls, the results are quite different. In four of nine pigeon eggs inoculated and developing, and in six of sixteen duck eggs, tumors were obtained. The inoculations were made into the

[6] Rous, *loc. cit.*

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membranes alone, and the growths developing there (figure 12) resembled those arising in similar situations in Plymouth Rock embryos. When portions were transplanted to adult pigeons and ducks respectively, no tumor resulted. In the eggs of an alien variety of chicken (Brahma), the growths were also similar to those in Plymouth Rocks.

These observations may be thought to lose in importance because of the short period (seven to ten days) during which the development of the tumor was noted, since in alien, adult fowls a brisk, temporary growth of the implanted tissue is often seen. But in alien adults, even in so short a time, an immense accumulation of lymphocytes takes place about the graft which itself shows well marked degenerative changes. In the embryo, neither phenomenon is present. The growing tissue appears "healthy," and about it small round cells do not collect, despite the fact that many such cells are present in the circulating blood.

RESISTANCE OF THE EMBRYO.

The question arises as to whether some individual chick embryos, like some adult hosts, possess a natural resistance to the tumor. Among 147 chick embryos that continued to develop after inoculation, 39 failed to show any tumor; and we at first supposed this finding to indicate a natural resistance, and referred to it as such in our preliminary note.[7] But an extensive search has failed to disclose, in any instance, those histological signs about and within the growing tumor which, in the adult, are associated with resistance of the host. Because of this total lack of evidence of partial resistance, we now believe that the non-appearance of the sarcoma in inoculated chick embryos is due entirely to chance causes. An analysis of the cases supports this idea. In seventeen of the thirty-nine, the inoculation was done at a very early period, according to the uncertain method already described; while in nine others, a Berkefeld filtrate alone was inoculated; and in five, dried material; leaving but eight that gave negative results after implantation with fresh tumor material, as compared with 108 positive results from inoculations of all sorts. Once the tumor has established itself, no

[7] Peyton Rous and James B. Murphy, *Jour. Am. Med. Assn., loc. cit.*

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effectual resistance is opposed to its growth by the chicken embryo, or even by the pigeon or duck embryo.

RELATIVE RAPIDITY OF GROWTH IN THE EMBRYO.

Bashford[8] first showed that young animals are more susceptible than adults as hosts for transmissible tumors; and Buschke[9] has since reported that this susceptibility extends even to the newly born. One of us has made independently many comparative observations on adult mice and mice one or two days old, which confirm Buschke's statement.

A number of complicating circumstances enter into the comparison of results in embryo and adult hosts, as can be well illustrated from the present work. The growth that follows inoculation of a minute bit of sarcomatous tissue into a twenty-four or forty-eight hour chick embryo is found, seven days afterwards, to be very small. But so too, for that matter, is the host nourishing it. The graft implanted in a membrane of an older chick grows vigorously. But here there is a notable absence of the mechanical restraints met with in adult hosts. Very much more tumor tissue, relatively speaking, is injected into embryo hosts than into adults, while, on the other hand, the actual amount is considerably less. These factors and many others complicate the comparison.

The findings in embryos injected at the tenth day are perhaps best compared with those in adults. Here, whether in the membranes or in the embryo's body, the growth of the tumor is excellent. The actual size of the masses formed is not behind that observed within the same period in adults, a fact that gains in significance when the small size of the host and the small amount of implanted tissue are recalled. Several counts have shown that in the tumors of the embryo more cells, on the average, are in process of division at one time than in those of adults (table I), while, as already stated, there is about the tumor a total absence of those histological signs of resistance so frequent even in susceptible adults. Altogether, chicks inoculated at the tenth day of incubation seem more favorable to the tumor growth than adult fowls.

[8] E. F. Bashford, J. A. Murray, M. Haaland, and W. H. Bowen, *Scientific Reports of the Imperial Cancer Research Fund*, 1908, No. 3, 265.

[9] A. Buschke, *Berl. klin. Wchnschr.*, 1911, xlviii, 215.

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TABLE I.

Relative Number of Dividing Tumor Cells in Adult and Embryo Hosts.

	Adult.			Embryo.			
	Mitotic, per cent.	Amitotic, per cent.	Total number dividing, per cent.	Total number dividing, per cent.	Mitotic per cent.	Amitotic per cent.	
Primary (from filtrate)	2	18	20	52	3	49	Liver.
Primary	4	42	46	70	1	69	Chest wall.
Primary (from dried material)	2	28	30	64	4	60	Membrane
Primary	3	20	23	67	3	64	Membrane.
Metastasis (ovary)	1	26	27	57	1	56	Membrane
Metastasis (liver)	3	44	47				
Metastasis (lung)	2	24	26	40	13	17	Membrane (pigeon).

Counts of one hundred cells at all stages of division.

We see no reason why this conclusion cannot be extended t embryos at earlier periods of development. The less strikin results in these must certainly be laid in large part to the method o inoculation, and to the relative unfitness of the host to nourish th implanted tissue, owing to its small size and simple structure.

SUMMARY.

The direct inoculation of a sarcoma of the fowl into the develop ing chick embryo or its membranes has yielded growths in man cases. The best results have been obtained with grafts of the livin tumor tissue, but, as in the adult, growths can be engendere with dried tissue or with the Berkefeld filtrate of a tumor extract When living tumor tissue is used, an actual transplantation occurs.

The neoplasms developing are spindle-celled sarcomata, remark ably uniform in structure, and similar to those in the adult fow except that in the embryo the neoplastic cells are often extremel long and slender, and the structure of the growth is very loose. Th membranes adapt themselves in a remarkable way to the support o the tumors. In them, the growth is seldom invasive; and whil regional metastases are occasionally seen, none occur by the blood

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stream, despite the predilection of the growth for this path of distribution in adult hosts. In the more resistant structures of the embryo itself, an invasive extension of the sarcoma occurs. Growths originally in the yolk-sac outside the chick may be carried into the latter during the course of development. Secondary growths in the viscera may cause the death of the host some weeks after hatching.

In order to produce tumors in the embryo, the sarcoma cells or the agent engendering the growth must be brought into a direct association with the mesodermal tissues. This necessity is responsible for interesting differences in the location of the growths in the various membranes.

The sarcoma will grow in the membranes of pigeon or duck embryos, whereas in adults of these species it will not do so; and in chicken embryos of different varieties, it grows uniformly well, a finding not obtained in adults. In embryo hosts of all the sorts mentioned, there is a total absence of the cellular reaction which in adults indicates resistance to the tumor's development. Relatively speaking, the embryo seems much more favorable than the adult as a host for the sarcoma.

EXPLANATION OF PLATES.

PLATE 17.

FIG. 1. A tumor in the outer allantoid membrane of an eighteen day chick. The growth is situated at the point where tumor tissue was implanted seven days before. It is sessile on the inner surface of the membrane. Note the large blood-vessels supplying it. The longest diameter of the mass measures 1.5 cm.

FIG. 2. Cross-section of a tumor, smaller, but otherwise similar to figure 1. It is a spindle-celled aggregate, attached to the membrane by a narrow base, covered by a single layer of endodermal cells, and supplied by vessels with thin walls. Extensive hemorrhage has occurred into the right half of the growth.

PLATE 18.

FIG. 3. Diagram adapted from "The Development of the Chick" by F. R. Lillie (Chicago, 1908). The chick and its membranes at the twelfth day of incubation are represented in cross-section. The mesodermal layer is given in red, and dotted lines show the direction taken by the injection needle. The distribution of the tumors in the various membranes is given in cross-hatched red.

All. and *Am.* = fused allantois and amnion; *Am. C.* = amniotic cavity; *Am.* = amnion; *E. E. B. C.* = extra embryonic body cavity; *Ect.* = ectoderm;

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Mes. = mesoderm; *Ent.* = entoderm; *All. I.* = inner allantoid membran
All. C. = allantoid cavity; *Y. S.* = yolk-sac.

FIG. 4. Multiple nodules in the extra-embryonic body cavity, as seen throu its wall. The embryo was at its nineteenth day of incubation, and the inocul tion had taken place on the eighth day.

PLATE 19.

FIG. 5. Sarcoma in the soft tissues of the leg of an embryo at the eighteen day of incubation, and the eighth day after implantation.

FIG. 6. Sarcomatous masses on the surface of the right auricle, and t right side of the thorax, respectively. Both are in the track of the injecti needle. Chick at the eighteenth day of incubation, and the seventh day aft inoculation.

PLATE 20.

FIG. 7. Beginning growth in the outer allantoid membrane, four days aft inoculation. The glistening, gray nodule projects sharply on the membran inner surface. The photograph is marred by reflections, seen as pale streaks the membrane and as an irregular, white area to one side of the tumor itself.

FIG. 8. Edge of a sarcomatous mass in the outer allantoid membrane. T tumor is made up of spindle cells in a loose network. About it there is cellular reaction. From an egg at the eighteenth day of incubation and t eighth day after inoculation.

PLATE 21.

FIG. 9. Cross-section of a sarcomatous nodule in the outer allantoid mem brane. The cells are of very attenuated spindle form. Many are in process amitotic division.

FIG. 10. Implantation nodule on the mesoderm lining the extra-embryon body cavity.

PLATE 22.

FIG. 11. Marginal portion of a "rice-body" found in the extra-embryon body cavity of an embryo at the eighteenth day of incubation and the seven day after inoculation. The mass consists entirely of tumor cells of very atten ated form, without blood-vessels or endo- or ectodermal covering.

FIG. 12. A portion of a sarcoma in the outer allantoid membrane of a duc embryo at the seventeenth day of incubation, and the seventh day after inocula tion. The growth is similar to those in the chick.

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FIG. 1.

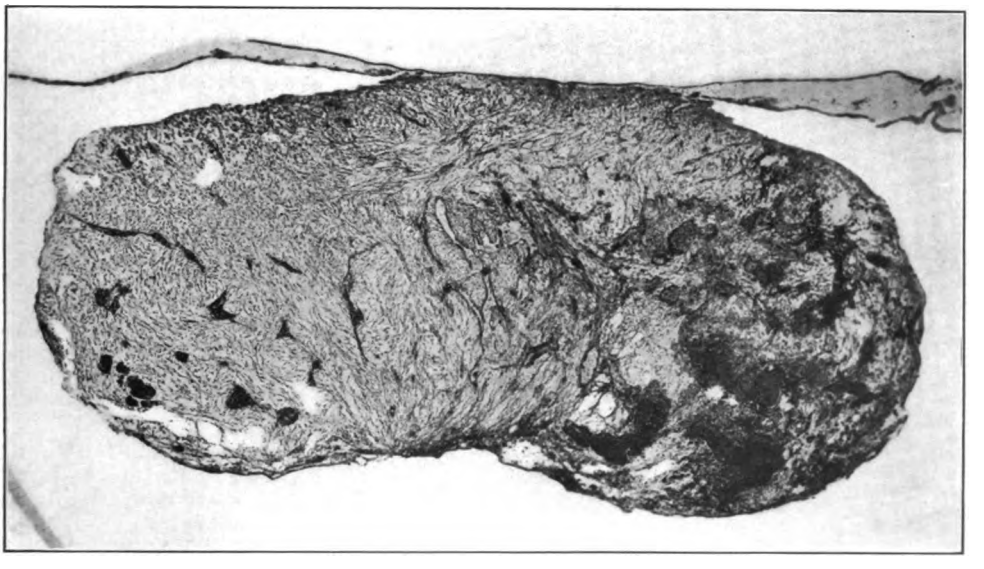


FIG. 2.

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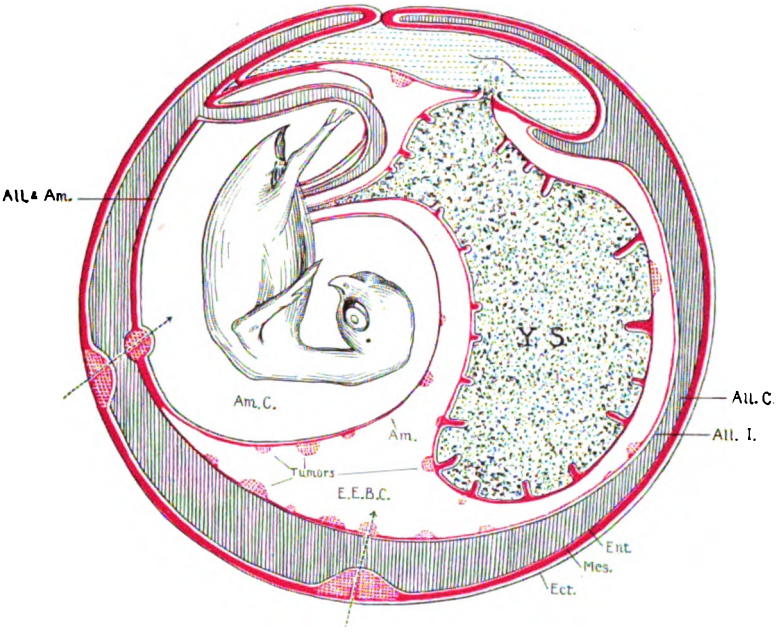


FIG. 3.

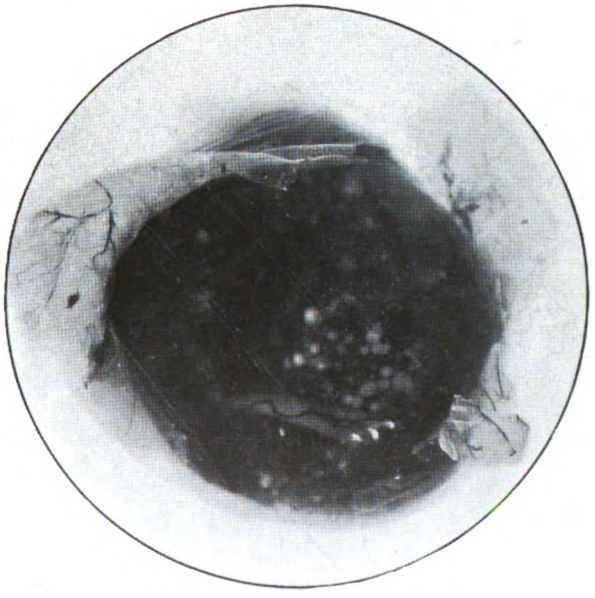


FIG. 4.

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FIG. 5.



FIG. 6.

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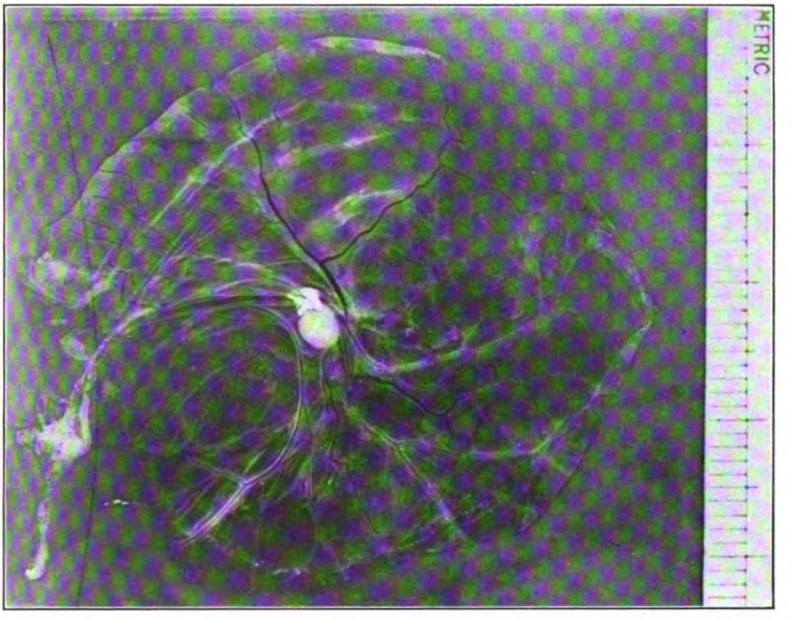


FIG. 7.

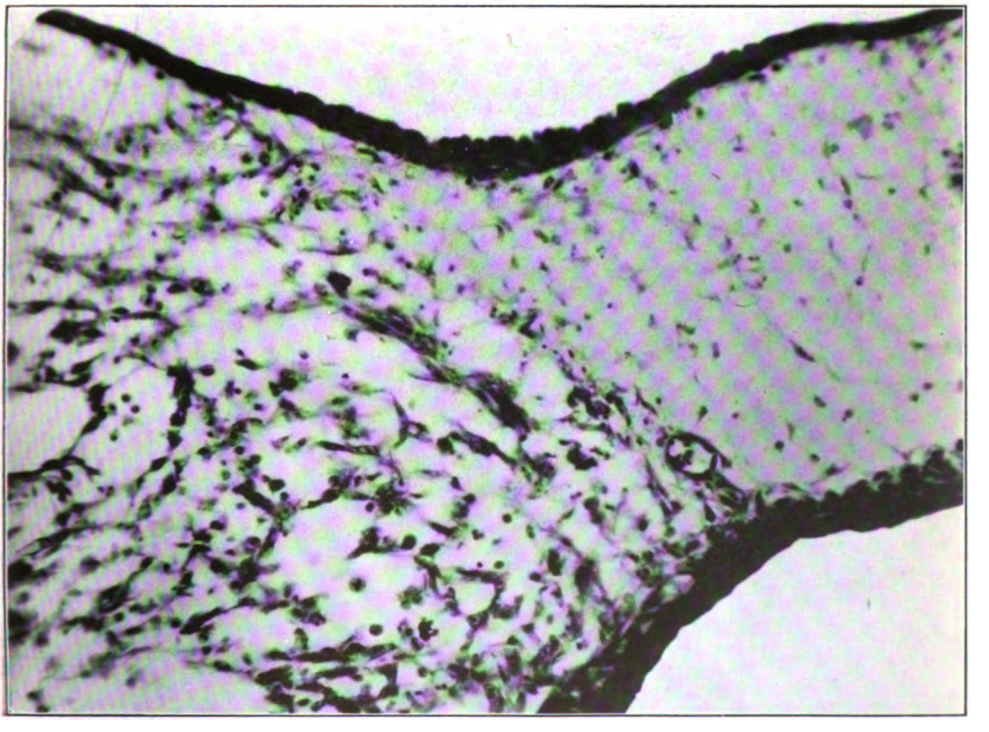


FIG. 8.

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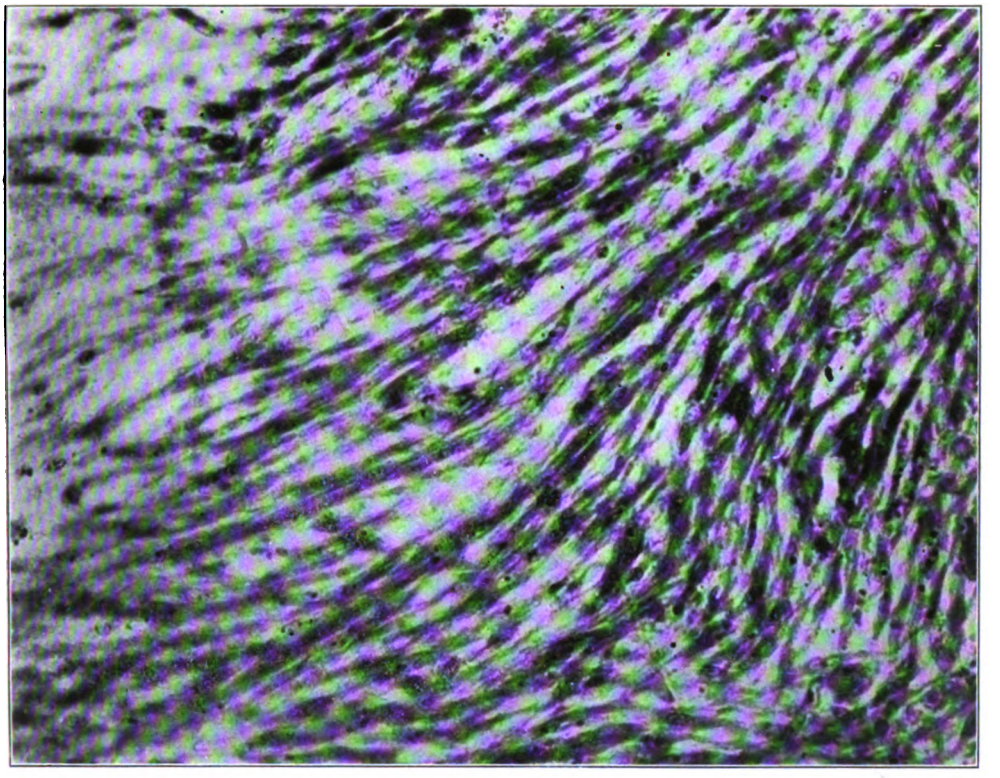
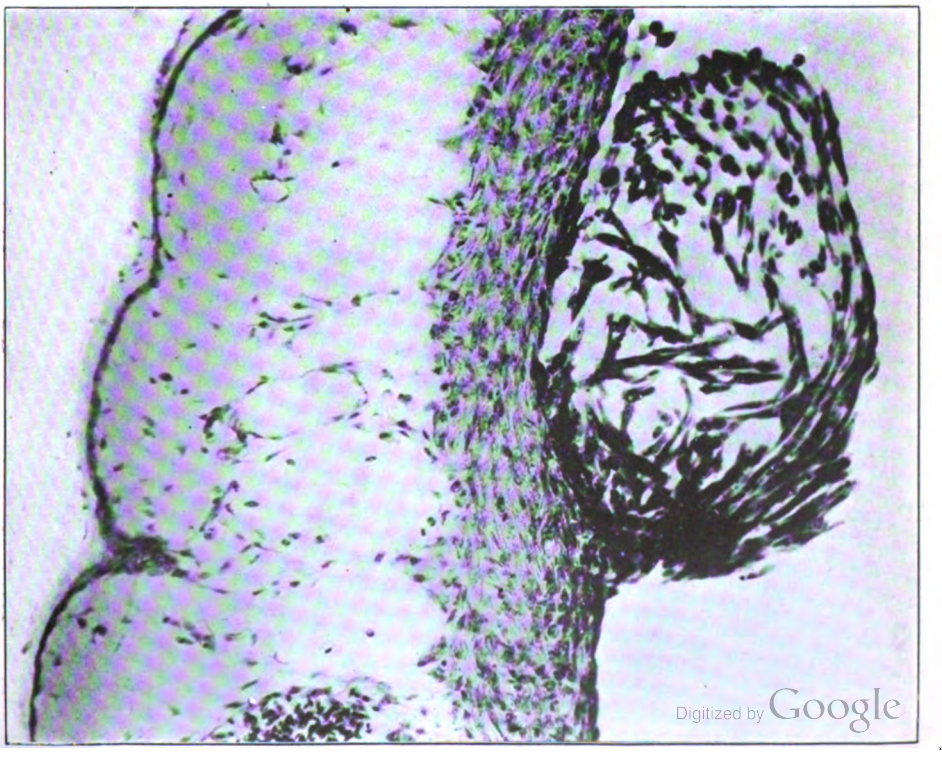


FIG. 9.



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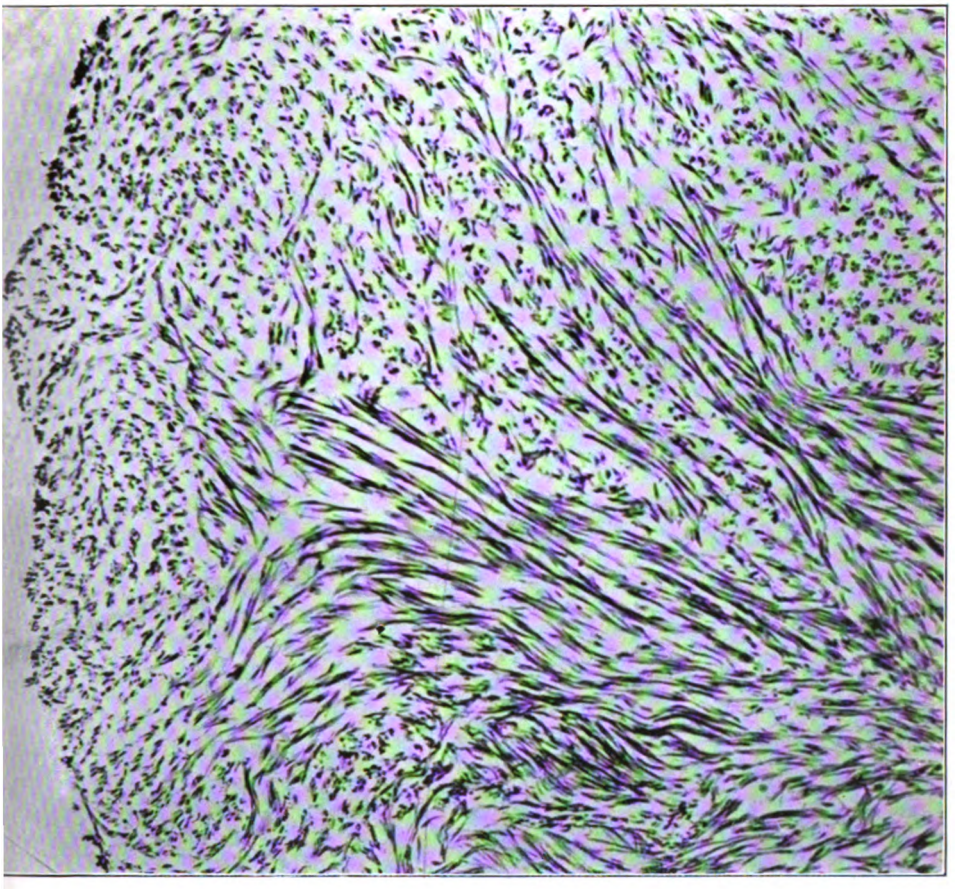
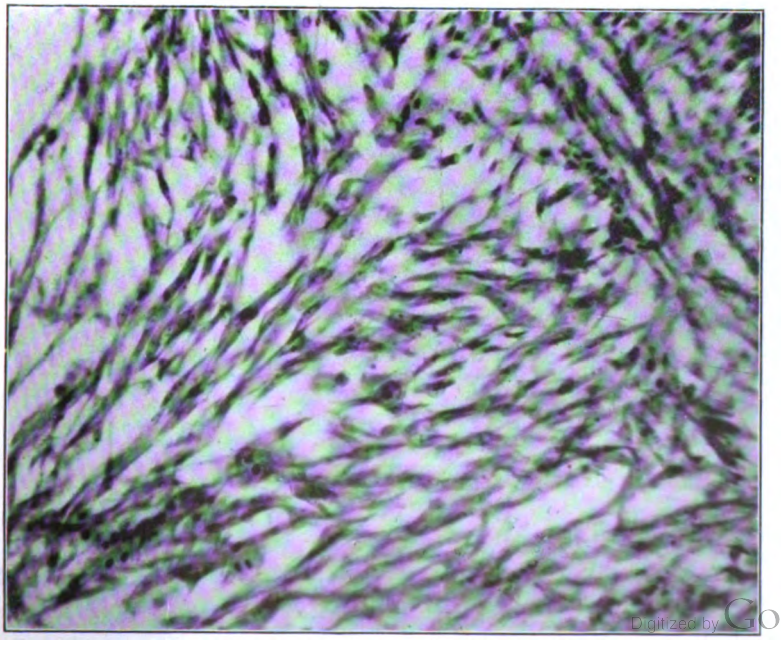


FIG. 11.



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EXPERIMENTAL PNEUMONIA BY INTRABRONCHIAL INSUFFLATION.*

BY R. V. LAMAR, M.D., AND S. J. MELTZER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

INTRODUCTION.

The most reliable evidence of an etiological relationship between a given microörganism and a definite disease is the experimental production of the disease by a pure culture of the microörganism. It is a quarter of a century since the organism which is now generally termed pneumococcus was brought into etiological connection with lobar pneumonia, and it is now generally accepted that this disease is, in most cases, caused by an infection of the lungs with the pneumococcus. Nevertheless, this assumption rests essentially on the statistical evidence that in most cases of lobar pneumonia the pneumococcus is recoverable from the lungs of the patients; while the more convincing evidence based upon the experimental production of pneumonia by inoculations with pure cultures is, in spite of numerous experiments, still lacking. If we are to judge from text-book and other statements, this fact is not generally appreciated. We shall therefore try to bring it out by a brief analysis of the data contained in the literature.[1]

We can omit the numerous experiments made in various ways with pure and impure cultures of the pneumococcus, the purpose of which was to ascertain the pathogenicity, and which led in most instances to septicemia, and only incidentally and exceptionally set up at the same time a pneumonia. We shall restrict our reference to those experiments that had as their exclusive object the production of pneumonia. Three methods of introduction of the pure cultures of the pneumococcus into the lungs were employed for this purpose: direct injection

* Received for publication, December 16, 1911.

[1] A recent paper by Wadsworth, entitled "Experimental Studies on the Etiology of Acute Pneumonitis" (*Am. Jour. Med. Sc.*, 1904, cxxvii, 851), contains a review of the literature and a bibliography of 198 references.

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of the culture into the lung tissue through the thoracic wall, intratracheal injection, and inhalation of the culture. The failure of the last named method is generally admitted, although several investigators believed that an occasional positive result was obtained. It is different with the other two methods. Gamaléia[2] reported that by injection of pneumococcus cultures through the chest wall directly into the lungs of dogs and sheep he obtained constantly pneumonic lesions, and Tchistovitch[3] stated that by intratracheal injections of cultures into dogs he succeeded in seven out of nineteen experiments, while Monti[4] claimed to have obtained constantly positive results by intratracheal injections in rabbits. These are the statements that are chiefly before the minds of those writers who consider that the experimental proof of the causation of pneumonia by the pneumococcus has been rendered. As a matter of fact, the several claims mentioned were put forward in the early years after the etiological connection of the pneumococcus with pneumonia had been expounded by Fränkel, but they were not substantiated by any subsequent experimenters. Kruse and Pansini,[5] who dismiss the experiments of Monti as insufficiently described and untrustworthy, failed to confirm the experiments of Gamaléia. Referring to the experiments of Gamaléia, Welch[6] says: "They are not described with sufficient detail to permit critical judgment, nor have they been satisfactorily confirmed by other investigators. Many inoculations of cultures of virulent pneumococci into the trachea and lungs of dogs have been made in my laboratory by Dr. Canfield and myself, but in no instance were we able to produce an inflammation of the lungs which we were willing to identify with acute lobar pneumonia as found in human beings. In the majority of our experiments there was no appreciable effect." Welch states further: "The domestic animals are not subject to a form of pneumonia etiologically and anatomically identical with croupous pneumonia of man. Bacteriologists are not always pathologists and the bare statement that the pneumonia produced experimentally is in all respects identical with acute lobar pneumonia in human beings should be received with caution." With the exception of that of Wadsworth, in the intervening nineteen years no investigation embodying a larger measure of success in the production of pneumonia in animals has appeared. This failure was, however, not utilized as proof against the claim that the pneumococcus is the cause of lobar pneumonia in man. Indeed, the failure to produce successfully the disease in animals was explained either by the assumption that the experimental animals employed are not susceptible to exactly this form of disease; or that besides the presence of the pneumococcus in the lungs there must coëxist other factors in the host that are indispensable for the development of the typical form of lobar pneumonia. The latter hypothetical notion was tested in several series of experiments which need not here be dwelt upon. It suffices to state that no well defined condition has thus far been discovered with the aid of which it is possible, using any

[2] Gamaléia, *Ann. de l'Inst. Pasteur,* 1888, ii, 440.
[3] Tchistovitch, *Ann. de l'Inst. Pasteur,* 1890, iii, 285.
[4] Monti, after Kruse and Pansini (see reference 5).
[5] Kruse and Pansini, *Ztschr. f. Hyg. u. Infectionskrankh.,* 1892, xi, 337.
[6] Welch, *Bull. Johns Hopkins Hosp.,* 1892, iii, 125.

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method of inoculation, to produce in animals, with fair constancy, definite lobar pneumonia. The assumption that experimental animals are not subject to lobar pneumonia is based upon the idea that some animals are so highly susceptible to the infective action of the pneumococcus that an inoculation of the lungs leads to a fatal septicemia without causing any local reaction in the lungs; while others are equally refractory to infection, perhaps by reason of the capability of the tissues of their lungs to destroy the invading organisms without the aid of any inflammatory reaction whatever. This conception was the starting point that enabled Wadsworth[7] to develop a method by which he succeeded in producing pneumonia successively in eleven rabbits. Rabbits are highly susceptible to the action of the pneumococcus, and the inoculation into the lungs of virulent cultures leads to a fatal septicemia without causing pneumonia. Wadsworth reduced experimentally this susceptibility by previously immunizing, in some degree, the rabbits, after which he injected pure cultures of pneumococcus of moderate virulence intratracheally. The positive outcome of the experiment would appear to depend upon an exact adjustment between the virulence of the microörganism and the susceptibility of the animal—a condition difficult to control. Although Wadsworth's experiments were reported about eight years ago, they appear not to have been repeated and confirmed.

In a preliminary note,[8] we reported upon our efforts to produce pneumonia in dogs by injecting cultures of the pneumococcus through the trachea into the bronchi, and pointed out that there may be produced regularly in this manner an extensive, evenly distributed, fibrinous pneumonia affecting the whole, or almost the whole, of one lobe or even two or more lobes of the lungs. The experiments were continued, and the present paper contains a more detailed report of the results achieved, as well as a consideration of their significance.

EXPERIMENTAL DATA.

Methods.—All the experiments were made upon dogs. The method consists in injecting pure cultures of the pneumococcus or other organisms into the lungs through a catheter or small stomach tube which is introduced through the mouth, larynx, and trachea into a bronchus by the insufflation procedure of Meltzer and Auer.[9]

The procedure is as follows: In a deeply etherized dog the mouth is kept wide open by means of a large wooden gag, the tongue is pulled out by means of hemostatic forceps, and then the median glosso-epiglottic frenum is grasped with a curved forceps and gently pulled forward. The now clearly visible pos-

[7] Wadsworth, *loc. cit.*

[8] Lamar and Meltzer, *Proc. Soc. Exper. Biol. and Med.*, 1909–10, vii, 102.

[9] Meltzer, *Med. Rec.*, 1910, lxxvii, 477; *Jour. Am. Med. Assn.*, 1911, lvii, 521.

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terior aspect of the epiglottis presents an inclined plane into the concavity of which one end of the tube is placed. Under the protection of the left index finger the tube is now directed into the larynx and pushed down slowly and gently through the trachea until a resistance is met with. The inner end of the tube is now in a bronchus, and usually in the right bronchus. The smaller the diameter of the tube, the deeper it enters into the bronchi. The opening of the tube should be at the end and not on the side where it usually is in catheters. A pipette containing the liquid culture is now attached to the external end of the tube, and by means of a syringe attached to the pipette the culture is injected into the bronchus. The syringe is now taken off, the piston withdrawn, and the syringe again attached to the pipette. By the injection of air the culture is now driven still deeper into the bronchi. The tube is clamped and withdrawn, and the animal released from the table.

The pneumococcus employed in the experiments was obtained from the sputum of a patient suffering from lobar pneumonia, and it had the characteristics of what is commonly termed a typical pneumococcus,—occurrence in pointed pairs, possession of capsules, solubility in bile, and ability to ferment inulin. Its virulence had been exalted to a high degree and was maintained at a constant level, in so far as one may know from the methods in use, by regular "passage" in mice, for which 0.000,001 of a cubic centimeter was fatal. Plain broth made from beef adjusted to a reaction of +0.5 per cent. to +0.7 per cent. (phenolphthalein) and sterilized fractionally in live steam was used as the culture medium. In such a broth the pneumococcus grows profusely, preserves its virulence, and suffers a minimum of involution and the autolysis to which it is prone.

The quantity of culture injected varied according to the particular purpose of the individual experiment from 0.5 of a cubic centimeter to 25 cubic centimeters. Most often it was from 5 to 10 cubic centimeters.

Results.—While some of the animals died as a result of the pneumococcus infection, most were killed for anatomical and bacteriological examination of the lungs at varying intervals of time, from one day to several weeks, after the injection was made.

Apparently in accordance with the quantity of culture injected the disease has been mild, of moderate severity and followed by recovery, or very severe and terminated by death. Correspondingly in the one there has been consolidation of nearly all of one lobe of

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the lung with a concomitant dry pleurisy, while in the other, there was usually consolidation of more than one lobe, accompanied by empyema, pericarditis, and septicemia. For the sake of convenience and clearness composite type descriptions drawn from the many individual examples of each variety will be given.

Non-Fatal Effects.—In most instances the disease produced was of the milder type. Within a few hours of the injection of the smaller or moderate quantity of culture, the animal eats little or nothing, becomes quiet, and its temperature rises two or three degrees Centigrade. This condition continues for the remainder of the day. On the next day, there is still slight fever, food is still refused, but the animal appears to be better. On the third day, the clinical symptoms have already subsided by lysis, after which there are no further objective signs of illness. Yet, as will be shown later, if the animal is killed at this time, the inflammatory exudate is found to be little diminished, although resolution has begun. Cough was noticed in a few instances only, and merely for a short time after the injection.

This has been the usual course. In a few instances, however, slight fever persisted, or returned after the temperature had been normal for one day or longer. In these cases the anatomical and bacteriological examination revealed a complication due to secondary invasion of the distemper bacillus.

By examining the lungs of the animals that died and of those that were killed at daily intervals from one to twelve days after the injection, it has been possible to follow the character of the consolidation, the changes which take place in the exudate, and the fate of the pneumococci.

In most of the experiments the consolidation was confined to the right lung and usually occurred in the posterior lobe, occasionally in the internal or subcardiac lobes. The anatomical relation of the right bronchus to the trachea explains this selection. Sometimes consolidation existed in the left lung but almost always in association with consolidation in the right lung.

The course of the inflammation in the lung is rapid, and consolidation occurs quickly. Thus seven hours after the injection, which is the earliest observation secured, nearly complete consolidation affecting the greater part of one lobe was already present.

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At the end of twenty-four hours nearly the whole of the right posterior lobe is solid and airless except for a small area at the anterior superior angle. Here are found usually only congestion and some edema, the inflammation not progressing to the point of complete consolidation. Often the entire lobe is solid; nearly always when it is the internal one that is affected. Rarely the consolidation is confined to the left posterior lobe, and then, as in the right, the anterior tip is commonly spared. The visceral pleura of the consolidated lobe is dark red and has lost its luster. There is a general deposit of fibrin as fine granules, with here and there a few small flakes or even a fairly large sheet. The consolidated portion is uniformly firm and inelastic, the lobe not flattening from gravity when placed upon the board. Excised portions sink at once in water. The cut surface is dark red, not very moist, and somewhat granular. On firm compression only a moderate quantity of rather thick bloody fluid can be squeezed out, the tissue soon giving way with a ragged line of fracture.

The exudate consists mainly of well preserved polymorphonuclear leucocytes closely filling the alveoli. There are red blood cells free in the alveoli but they are usually relatively few in number and unequally distributed; some alveoli contain many, and others only a few. Occasionally many alveoli are filled with red blood cells. Fibrin is present throughout as fine threads and granules. At this early stage, as is the rule in sections made from inflamed tissue, it is not as prominent or apparently as abundant as a day or two later. The bronchi, themselves free from inflammation, are filled with the exudate from the alveoli. When the whole lobe is not consolidated, the composition of the exudate changes near the site which is only congested and edematous. Here serum begins to predominate over cells, the leucocytes are fewer, and there is some epithelial desquamation.

At this time pneumococci are seen in large numbers, capsulated, in pairs, and often in short chains. They lie extracellularly for the most part, free in the alveoli and finer bronchial vessels. Examples of phagocytosis are not numerous.

The lymph nodes related anatomically to the consolidated lobe are swollen, soft, and moist. The sinus contains many polymorpho-

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nuclear cells, and the germinal centers are prominently defined. There is often a mild degree of acute splenic tumor.

By the end of the second day the pleura is more dull and the quantity of fibrin is increased, often to the extent of shaggy patches. The consolidated portion of the lung is still firm, but it already begins to flatten from gravity and is less friable. Some of the polymorphonuclear leucocytes are disintegrating. In a few alveoli there are free large mononuclear cells, some of which have ingested polymorphonuclear leucocytes. Examples of this macrophage activity may be seen also in the vessels of the lymph plexus deep in the pleura.[10] The diplococci are fewer in number and many are now intracellular. Growth from the exudate is not so profuse as before.

On the fourth day, resolution is fairly inaugurated. The lobe is softer and no longer uniform in color and consistence, some parts being softer and of a lighter red color than others, or even grey. Nearly all of the pus cells stain poorly, the ratio of large mononuclear cells has increased, and a fair proportion of alveoli contain only, or largely, serum. Phagocytosis of the polymorphonuclear cells continues to a greater extent. Many examples of it are seen in the alveoli, in the lymph vessels, and in the peripheral sinus of the related lymph nodes, the swelling of which has subsided.

Usually at this time only a few diplococci may be observed, and they lie within the polymorphonuclear cells. Transplantations furnish slight growth.

From now on resolution proceeds rapidly but not uniformly throughout. It advances rather from the edge and circumference of the lobe and the anterior margin of the consolidated part toward the center, where it is slowest about the larger bronchial vessels, and the diaphragmatic surface. Sometimes by the end of the first week little of the exudate remains. Nearly always before two weeks have passed the lung has returned to the normal condition.

In several instances, resolution was not complete, there being left behind one or more small streaks or patches in the thin edges of the

[10] From the constancy of its occurrence and the many examples of it, this phagocytic activity of the large mononuclear cells, to the occurrence and significance of which in inflammation Opie (Harvey Lectures, 1909–1910, 192) has recently again directed attention, seems to be an important factor in the removal of the exudate.

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lobe where the persisting exudate was undergoing organization. In two instances, organization of the exudate was extensive, once affecting nearly the entire lobe. It is likely that organization is more or less dependent upon the secondary invasion of the exudate by the distemper bacillus, since in some instances, in the early stages, we have cultivated the bacillus of that affection. Later, as is customary in the spontaneous pneumonia of dogs which is prone to undergo partial organization, no growth is obtained.

ILLUSTRATIVE PROTOCOLS.

Dog 7.—Mongrel, male. Weight, 5.3 kilos.

March 23, 1910. 11 A. M. Temperature 38.6°. 11.30 A. M. Under ether anesthesia, injected intrabronchially 5 c.c. of a 20 hour plain broth culture of pneumococcus N. I. The animal recovers promptly and completely in a few minutes, as from simple anesthesia. 5.00 P. M. Quiet; seems ill; temperature 40.4°.

March 24. 10 A. M. Better, but not normal. Weight, 4.9 kilos. Temperature 38.8°. 11 A. M. Killed with ether.

Autopsy.—Performed at once. The posterior lobe of the right lung is completely consolidated for about four fifths of its extent. The remaining fifth at the anterior superior angle is congested and edematous. The surface of the consolidated portion is raised, dark red, moist, and glistens less than the other lobes of both lungs. Consistence is evenly firm. Excised portions sink at once in water. The cut surface is evenly dark red, very moist, and finely granular. Upon squeezing, there is much thick blood-stained fluid unmixed with air, and the cut bronchial vessels exude a viscid, reddish brown, semifluid material.

On the contiguous inferior surface of the right middle lobe is a thin, dark red streak following, and adjacent to, the main bronchial vessel. It contains air throughout. The remainder of the right lung, as well as the entire left lung, is normal in appearance.

The lymph nodes at the root of the right lung are swollen and soft; in section they are moist and translucent.

Smears of scrapings from the cut surface of the consolidated portion contain a great many well preserved polymorphonuclear leucocytes, a moderate number of red blood cells, a few large mononuclear cells, and a fair number of extracellular, pointed, Gram positive, capsulated diplococci. Smears from the congested and edematous anterior angle of the lobe show much serum, many polymorphonuclear cells, and many diplococci.

Cultures.—From the consolidated portion of the right posterior lobe: profuse growth of pneumococcus; pure.

From an opened bronchial vessel in the same portion: the same.

From the adjacent edematous portion: the same.

From the right middle, right upper, and left lower lobes: no growth.

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In sections from the consolidated portion, all of the alveoli are evenly filled with well stained polymorphonuclear leucocytes with a very few red blood cells here and there. At the edge of the consolidated area the alveoli contain fewer and fewer polymorphonuclear cells, more and more serum, and a good number of desquamated epithelial cells.

Dog 5 (New Series).—Weight, 10.5 kilos.

October 7, 1910. 10 A. M. Temperature 38.4°. 11 A. M. Under ether anesthesia, injected intrabronchially 5 c.c. of a 24 hour plain broth culture of pneumococcus N. I. Quick, complete recovery. 5 P. M. Quiet; temperature 39.8°.

October 8. 10 A. M. Temperature 39.5°.

October 10. 10 A. M. Temperature 39.5°.

October 11. 10 A. M. Temperature 39.3°.

October 12. 10 A. M. Temperature 39.5°.

October 13. 10 A. M. Temperature 39.1°.

October 14. 10 A. M. Temperature 39.2°. 11 A. M. Killed with chloroform.

Autopsy.—Performed at once. Both lungs are slightly emphysematous. The inflammatory change is confined to the posterior lobe of the left lung. The diaphragmatic surface of this lobe is dark red, rather dry, and shaggy in places from tags of fibrin. The subjacent lung tissue is solid and airless forwards for a distance of 1.5 cm., except along the superior margin and at the external angle, where it is only half consolidated. The irregularly light and dark red consolidated portion gives way anteriorly in an uneven line to grey and red lung tissue, very moist and containing much air until about the middle of the lobe, from where onwards to the anterior tip the tissue is only more moist than normally, being neither consolidated nor congested. The tip is bound firmly by a fibrinous deposit to the contiguous middle lobe and is superficially solid for an area of about 1 sq. cm.

The consolidation described first comprises about one fourth of the lobe. No bacteria are seen in smears; but there is a scant growth of pneumococcus in transplantations.

In sections the alveoli near the base of the lobe are fairly regularly filled with polymorphonuclear cells in various stages of disintegration. Farther forwards, serum and large mononuclear cells appear and come to make up most of the exudate, until, at the limit of the consolidated part, there is only a small quantity of precipitated albuminous material in the alveoli. Many of the large mononuclear cells contain one or more poorly staining polymorphonuclear cells.

At the base of the lobe fibrin is abundant on the surface of the pleura and within the subjacent alveoli. Farther forwards there is little.

Fatal Cases.—In the fatal cases the clinical course of the disease and the anatomical changes are quite different from what has just been described. As already stated, a certain relation was found to exist between the quantity of culture injected and the severity of the disease produced. Most of the deaths followed the injection of a large quantity of culture, and, conversely, the injection of a large quantity was usually fatal.

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The animals instead of beginning to improve on the day after the injection become worse; the fever persists; the animal does not eat, is prostrated, and dies in from two to four days after inoculation.

There is a corresponding difference in the anatomical changes. At least one lobe, and sometimes two or even three lobes are entirely consolidated. The pleural cavities contain a large quantity of bloody fibrino-purulent exudate, the pericardium is inflamed, and a septicemia exists. The exudate within the lung and in the pleural and pericardial cavities contains, as well as the blood, large numbers of capsulated pneumococci in pairs and short chains. There is an acute splenic tumor. Of the ten fatal cases, all except three presented serositis and septicemia. In two of the three instances where these complications did not occur there were several foci of necrosis in the consolidated lobes, with which a spore-bearing bacillus was associated; and death was probably due to this secondary effect.

Thus anatomically and bacteriologically the fatal type of the experimental disease resembles even more closely acute lobar pneumonia in man than the mild type.

ILLUSTRATIVE PROTOCOL.

Dog 16.—Weight, 4.5 kilos.

April 7, 1910. 10 A. M. Temperature 39°. 11.30 A. M. Under ether anesthesia, injected intrabronchially 8 c.c. of an 18 hour plain broth culture of pneumococcus N. I. Quick, complete recovery. 5 P. M. Moderately ill; temperature 39.5°.

April 8. 9.30 A. M. Worse; temperature 39.8°.

April 9. 10 A. M. Very ill; weight, 4.5 kilos; temperature 40.1°. 3 P. M. Found dead.

Autopsy.—Performed at once. The right pleural cavity contains about 20 c.c. of blood-stained, purulent exudate with many flakes and masses of fibrin. The pleural surfaces are bright red and shaggy in places from deposits of fibrin. The left pleural cavity contains no fluid, but there is congestion and a dry fibrinous exudate over the anterior lobe.

The anterior lobe of the left lung and the middle and internal lobes of the right lung are solid and airless. There are also two areas of consolidation in the right posterior lobe comprising about one fourth of its volume. The anterior lobe of the right lung is collapsed; the middle and posterior lobes of the left are emphysematous. The pericardial cavity contains 4 c.c. of bloody purulent fluid with a large clump of fibrin.

In smears from the consolidated lobes and the pleural and pericardial exu-

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dates, there are enormous numbers of pointed, capsulated, Gram positive diplococci in pairs and short chains. In the heart's blood there are also large numbers. Transplantations from these sources give profuse pure cultures of pneumococcus.

Sections from the anterior lobe of the left lung and the internal lobe of the right show alike an evenly distributed purulent exudate filling the alveoli completely. There are very few red blood cells in any alveoli. Nearly all of the alveoli contain fine granules of fibrin.

Statistical Data.—The series comprised forty-eight animals. Ten died, thirty-four were killed within from one to twelve days, and four, several weeks after the injection was made. Of the forty-four that died or were killed within twelve days of inoculation, forty-two were found to have pneumonia. In the remaining two the lungs were normal. One of the latter animals formed part of an experiment in which four were used to determine whether a large quantity of culture would prove constantly fatal. The four animals were injected one after another, each with fifteen cubic centimeters of the same lot of culture. Three of them became very ill and died in one and a half, one and a half, and four and a half days, respectively. In two, the whole, and in the third three fifths of the right posterior lobe was consolidated. All had empyema, pericarditis, and septicemia. It is from their protocols that the description of the fatal disease as given is partly drawn. The fourth animal was not ill, had no fever, and when killed on the eleventh day, the lungs were found to be normal. The other negative result consisted of an animal which received six cubic centimeters of culture and was killed on the fourth day afterwards.

It is quite certain that in the two failures the tube, instead of being introduced through the larynx, was by mistake pushed down into the esophagus.

The lungs of the four animals that were killed several weeks after the injection were normal, which finding is in agreement with the progress of resolution and the time required for its completion according to our description.

Pneumococcus mucosus.—Besides the typical pneumococcus, we have injected dogs in the same way with a virulent culture of *Pneumococcus mucosus* (Schotmüller's *Streptococcus mucosus*) recently grown from the spinal fluid of a case of meningitis. This organism

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produced virtually the same kind of extensive fibrinous consolidation as the typical pneumococcus, the only difference being that the *exudate was more moist and quite viscid.* This difference was probably dependent upon the inherent characteristics of the organism itself rather than upon any essential difference in the character of the exudate furnished by the host. When cultivated upon artificial media, the organism manifests the same character of greater moisture and viscosity of growth as compared with the pneumococcus.

Friedländer's Pneumobacillus.—Two experiments made in the same way with a culture of the Friedländer bacillus pathogenic for mice, resulted in the production of an evenly distributed consolidation affecting nearly the whole of one lobe. The consolidated part was of a lighter red color than that produced by the pneumococcus, even grey in places, less friable, and extremely moist and viscid—peculiarities which are described as characteristic of lobar pneumonia in human beings when caused by this organism.

Control Experiments.—In several control experiments, salt solution, broth, serum, and mixtures of serum and soap solution were injected in the same way as the cultures. In none did consolidation follow.

Likewise a number of experiments made with a pathogenic streptococcus and with the influenza bacillus, which are not detailed here as they are apart from the purpose of this paper, may be considered as controls, for these bacteria produced a kind of inflammation which could not be mistaken for, or confounded with, the consolidation caused by the pneumococcus.

We may mention, finally, that in a few of the animals respiration and heart action stopped during the preliminary etherization and the animals had to be resuscitated by means of intratracheal insufflation. The intrabronchial injection of the culture followed immediately on the resuscitation. This incident had apparently no unfavorable influence upon the course of the pneumonia; all the animals survived and the course of the disease was a mild one.

DISCUSSION.

By means of an intrabronchial insufflation of a pneumococcus culture we succeeded in producing lobar pneumonia in dogs. In forty-

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two successive experiments there was not a single failure. The failures in the two additional cases were due to errors in the technique, the tube being introduced into the esophagus instead of into the trachea. The anatomical and bacteriological findings in the successful experiments accorded essentially with those usually found in cases of fibrinous pneumonia of man. The consolidations were massive and lobar, and not patchy or lobular. As in croupous pneumonia, distinguished from the lobular and pseudolobar varieties, the inflammation was confined to the specialized terminal portions of the respiratory tract—the alveoli and infundibula; the mucosa of the bronchi remaining uninvolved. The interrelations of microörganisms, leucocytes, and the large mononuclear lymphocytes according to the stages of the inflammatory process, resembled those occuring in lobar pneumonia in man.[11] The findings in the fatal cases were strikingly similar to those in some fatal cases of pneumonia in man; such as pericarditis, empyema, septicemia. The mortality of the cases uncomplicated by secondary infections was at least as high as in lobar pneumonia in man: 7 in 41, or 16 per cent. At the same time, we ought to bear in mind that, among the non-fatal cases, animals are included which were killed on the first or second day, some of which, if left to themselves, might not have recovered.

However, in one respect the experimental pneumonia in our dogs differed from pneumonia in man, and that is in the clinical course of the non-fatal cases. In man, the temperature, as a rule, remains continuously high for a week or longer, and the disease terminates mostly by crisis. Furthermore, the critical termination of the clinical symptoms seems to coincide with the onset of resolution. In other words, the clinical symptoms in man seem to run parallel with the anatomical changes. This was not the case in the non-fatal cases of our experiments. While the pathological processes even in the milder cases were, in character as well as in duration, similar to those of pneumonia in man, the clinical course of the non-fatal cases was, as compared with the pathological processes, short and mild; fever and recognizable malaise subsided by lysis in a day or two.

[11] One distinguishing feature not itself essential to the process should be noted. The transition from red to grey hepatization was wanting.

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This discrepancy may be explained by one or two assumptions. Doubtless the pneumococcus employed for injection was not "adapted" to dogs as the pathogenic pneumococci, producing pneumonia spontaneously, are adapted to man. Whether such an adaptation could be secured by successive passages so as to lead to a disease simulating more closely in clinical aspects the human affection, is a matter of speculation. It may be, furthermore, that dogs, unless overwhelmed with fatal doses of the virus, are less apt to respond with general reaction—perhaps just because they respond with a strong local reaction of the lungs.

However, whatever the cause, it does not affect the significance of the results, which show that by an intrabronchial insufflation of a pneumococcus culture, pulmonary inflammation may be produced, which from a pathological point of view resembles closely lobar pneumonia of man.

We believe, therefore, that these results furnish the first unmistakable experimental evidence of an intimate etiological relationship between the pneumococcus and lobar pneumonia. Attention is invited to the fact that the dogs were not prepared in any manner, or specially selected. Nevertheless, they responded to the infection with a definite pneumonic reaction. Hence the causation of the pneumonia and its outcome depended entirely upon the invading organism, its numbers and virulence; and the condition of the host was an inconsiderable factor in the result.

The essential reason for our experimental success, we believe, is contained in the fact that the injected quantity of the culture was sufficient to obliterate a group of small or even larger bronchi.

In this manner, the lumina of the bronchi and the corresponding alveoli were converted into closed cavities or semi-solid masses which contained numerous pneumococci in close proximity to the pulmonary tissue. It seems to us that the simple presence of pathogenic bacteria on a free non-denuded surface does not readily call forth a local reaction and an infection. The injections of small quantities of a culture into the trachea usually failed to call forth a pulmonary reaction, because the small quantity of the injected culture had to spread over large areas; and even if some of it reached certain bronchi or alveoli, the quantities were too minute to

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cause the obliteration of even very small pulmonary areas. Pathogenic bacteria call forth an inflammatory reaction only when they have a chance to settle and develop their activities in the meshes of the various tissues or in closed narrow tubes and the small cavities of the body. The simple presence of pneumococci in the mouth does not produce an infection. Neither would these organisms be capable of producing an infection and inflammatory reaction in the pulmonary alveoli if they were free there or imbedded in masses too minute to obliterate these spaces. A quantiy of six cubic centimeters of a broth culture suffices apparently for dogs, to obliterate a fairly large number of bronchi and thus give the organisms therein a chance to cause a fairly extensive inflammatory reaction. But in these cases the local and general defensive elements of the tissues and the fluids of the body were sufficient to antagonize the infective and toxic action of the relatively large number of pathogenic organisms contained in this quantity. When, however, larger quantities of the culture were injected and larger areas of the lungs were converted into battle grounds, the local and general defences often failed to resist the too numerous invaders, and a lethal result was often produced. According to this view, the question of experimental success does not depend so much upon the alteration of the power of resistance of the invaded individual, as upon the opportunity offered to the invading pathogenic organisms for intrenchment in the invaded territory.

SUMMARY.

The numerous attempts made in the last twenty-five years to reproduce lobar pneumonia in animals practically failed in all instances.

By intrabronchial insufflation of pure cultures of pneumococcus in dogs, we produced experimental pneumonia successively in forty-two cases, with a mortality of at least 16 per cent. The fatal cases resembled closely lobar pneumonia in man. In the non-fatal cases, the pathological and bacteriological findings were again in accord with the findings in man. Clinically, however, the cases of non-fatal experimental pneumonia run a milder and shorter course than in man.

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In a few instances, lobar pneumonia has been produced experimentally also with the *Pneumococcus mucosus* and with Friedländer's pneumobacillus. The anatomical findings in these experiments have shown some characteristics agreeing with the findings in the pneumonias of man produced by these organisms.

The quantity of the injected culture seemed to have a definite influence upon the outcome of the disease; in the fatal cases larger quantities of the culture had been injected.

The animals were neither selected nor prepared in any manner. The experimental success did not, therefore, appear to depend upon the degree of resistance of the individual hosts.

It is suggested that the uniformly successful results of the experiments were due to the obliteration of a large number of bronchi by the injected culture, through which mechanical effect a favorable opportunity was provided the pneumococci to develop and display their pathogenic activities consisting in the calling forth of a characteristic local, more or less effective, widespread, inflammatory reaction of the lung tissue.

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MORPHOLOGICAL AND PATHOGENIC VARIATIONS IN TREPONEMA PALLIDUM.*

BY HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATE 29.

Now that *Treponema pallidum* can be cultivated artificially, it becomes readily possible to consider the question of differences in strain among the species that may amount to racial variations. Many observers have doubtless noted differences in the morphology of the pallida in living and dead film preparations and possibly even in stained tissue specimens. It would scarcely be possible to divide the species into strains on the basis of such superficial characters; but now that artificial cultivation has been accomplished, the question can be approached with adequate means for its solution.

During 1910 and 1911, while carrying simultaneously ten different strains of the pallidum through the testicles of rabbits for many generations, I was struck with the fact that certain strains appeared thicker than others. Most of the pallidum strains in my collection showed the thickness, length, and curves ordinarily recognized for this organism (figure A). On the other hand, two of the ten strains were distinctly thicker (about 0.3 of a micron), and one was thinner (about 0.2 of a micron) than the average (about 0.25 of a micron). The curves of the heavier type were quite typical (figure B), while those of the thinner strain were somewhat shallower and set more closely, except the portions towards the extremities, which were again quite typical (figure C). These characteristics were retained unmodified throughout the numerous transfers from one rabbit to another within a period of over a year. Further, it was noted that the thinner strains possessed greater agility than the heavier ones.

As regards the production of testicular lesions in the rabbit, the

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different types of the pallidum showed different effects. Seven strains of typical morphology produced a diffuse induration and swelling, usually within three weeks, that progressed steadily for five or six weeks, when spontaneous retrogression set in. The thicker type, including two strains originally transmitted to rabbits from cases of protracted chancres on the lip and prepuce, respectively, did not, as a rule, produce any change in the inoculated testicles until about five or six weeks after inoculation, when small, localized, hard nodules developed. The nodules continued to increase in size slowly and remained sharply demarcated from the surrounding soft tissue, attaining their maximum size in eight to ten weeks. Unlike the effects caused by the typical strains, the nodules produced by the heavier ones were cartilaginous in consistence and contained a greater quantity of mucin. The thinner strain that was transmitted originally from a mucous patch in a case of malignant syphilis, always caused diffuse testicular swelling, within ten to fourteen days, that progressed rapidly for five to six weeks.

Of these three forms of the pallidum, namely, thicker, average, and thinner types, I have succeeded in cultivating from the testicle of the rabbit one specimen of the thinner, five of the average, but none of the thicker variety. The pure cultures of both types were found to produce typical syphilitic orchitis in the rabbit.[1]

During 1911, I have cultivated six strains of the pallidum directly from human syphilitic lesions including chancres, condylomata, and skin papules.[2] I wish now to report briefly upon certain morphological differences that I have observed in different cultures of the pallidum obtained directly from human sources.

Of the six strains cultivated directly from human cases, three exhibit the perfectly typical morphology, while one is thicker and two are thinner than the average. The average or typical strains all came from chancres, the thicker one from a chancre, and the thinner strains were respectively from a condyloma and a chancre in which they were associated with a regular strain.

Under cultural conditions, every pallidum strain is subject to some degree of morphological change through which very long filaments, seldom seen in syphilitic tissues, with shallower or compara-

[1] Noguchi, *Jour. Exper. Med.*, 1911, xiv, 99.
[2] Noguchi, *Jour. Exper. Med.*, 1911, xv, 90.

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tively few curves, appear. But, when provided with optimum conditions, the morphology approaches the normal to such an extent that no difference can be found between the cultivated and the natural pallidum occurring in the tissues. When, however, comparison of the three main types of the pallidum is made under optimum cultural conditions, the definite variations are easily observed. Even under less favorable cultural conditions, in which some irregular forms are apt to appear, the type characteristics between the thinner and the two thicker forms can still be clearly made out. Under distinctly unfavorable conditions, the differences between the thicker and the regular types becomes less evident.

In spite of the morphological variations mentioned, all the strains grow, under common conditions, with identical macroscopic appearances. Further, they are all pathogenic for certain species of monkeys.

In morphology, the thinner pallidum presents marked resemblance to *Treponema microdentium*,[8] especially when the microdentium has been under cultivation for two or three weeks; but in macroscopical characters of growth, in respect to non-requirement of fresh tissue for multiplication, and, especially in the occurrence in old cultures of a characteristic fetid odor, the microdentium is sufficiently differentiated from this strain of the pallidum.

SUMMARY.

When many cultures of *Treponema pallidum*, whether obtained from the testicular lesions produced in rabbits or directly from human cases of syphilis, are compared, certain definite differences in morphological character become apparent. The different specimens can be divided into thicker and thinner forms or types, and an average or normal form or type. The last is the common or most frequent variety, but the other two occur with sufficient frequency and retain their characters with such constancy as to constitute distinct varieties. Indeed, two of the varieties—the average and the thinner—occurred in association in a chancre and were separated afterwards in cultures. The gross cultural properties of the three varieties present no points of distinction.

[8] Noguchi, *Jour. Exper. Med.*, 1911, xv, 81.

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The lesions caused in the testicle of the rabbit differ according to the variety inoculated, and consist either of a diffuse or of a nodular orchitis. This is a highly important distinction, and if, in the study of a still larger number of specimens of pallida, it is maintained, it is capable of throwing light on certain important clinical features of the human syphilitic disease.

The thinner variety of *Treponema pallidum* resembles in morphology *Treponema microdentium,* from which it is unmistakably distinguished by cultural characters.

The morphological and pathogenic variations in cultures of the pallidum may constitute racial differences within the species.

EXPLANATION OF PLATE 29.

FIG. A. Regular type of *Treponema pallidum.* Schematic reproduction.

FIG. B. Heavier type of *Treponema pallidum.* Schematic reproduction.

FIG. C. Thinner type of *Treponema pallidum.* Schematic reproduction.

FIG. D. Various forms of the thinner pallidum in a pure culture. Schematic reproduction.

FIGS. 1 and 2. A long form of the regular type of the pallidum. From a pure culture thirty-two days old. × 1,400.

FIG. 3. Average forms of the regular type in a pure culture two weeks old. × 1,400.

FIG. 4. Agglomeration of the pallidum of the regular type. From a pure culture four weeks old. × 1,400.

FIG. 5. A pallidum of the thinner type. From a pure culture three weeks old. × 1,400.

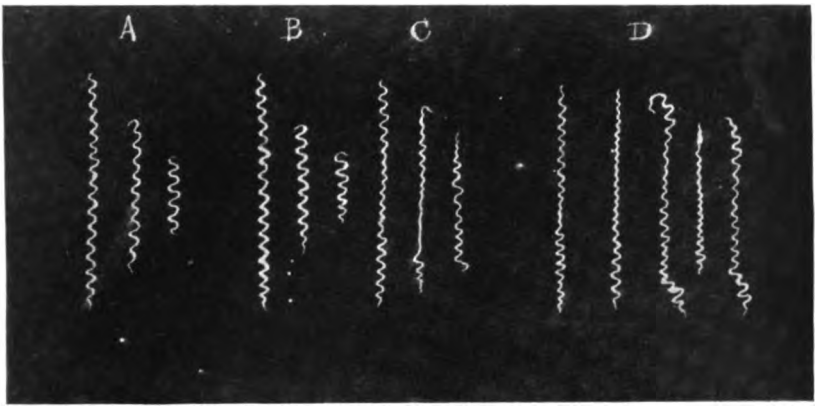
FIG. 6. A pallidum of the thinner type. From a pure culture three weeks old. × 1,400.

FIG. 7. A pallidum of the thinner type showing deeper curves near one end. × 1,400.

FIG. 8. A group of pallida from a pure culture of the thinner type. × 1,400.

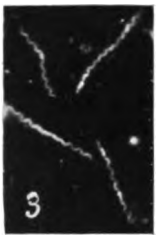
FIGS. 9 and 10. Masses of entangled pallida from two different pure cultures of the thinner type. × 1,400.

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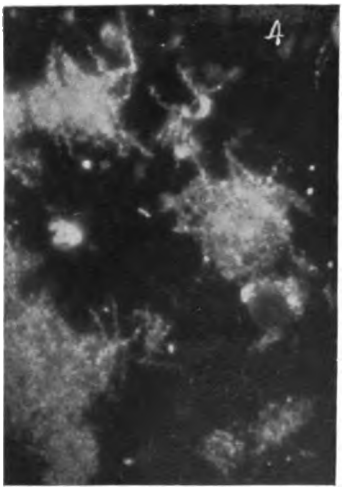


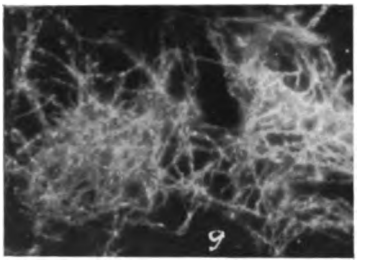




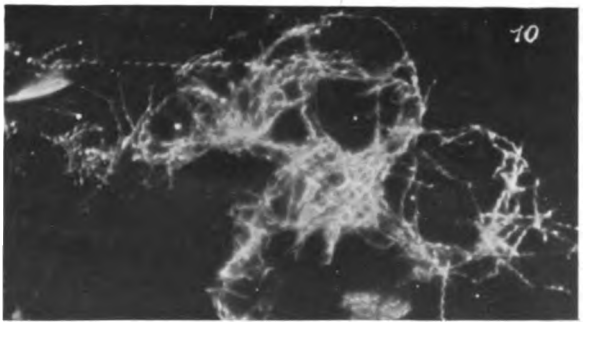












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CHEMISTRY OF THE DOG'S SPLEEN.[1]

BY HARRY J. CORPER.

(From the Department of Pathology, University of Chicago.)[2]

(Received for publication, December 3, 1911.)

In conjunction with experimental work on the histological and chemical changes in the spleen during autolysis, results of which are to be published later, it was found desirable to analyze spleens and also to study their purine enzymes. The results of these studies are given in this paper.

The dog's spleen has never been analyzed in a complete manner chemically, but has frequently been used for the study of its enzymes and individual elements. The spleen of man and other animals has, however, been studied more fully. The water content of the spleen is given as about 78 per cent,[3] 70 per cent to 77 per cent,[4] Schulz[5] finding 79.5 per cent. in human spleen. 12.5 per cent of the spleen is blood content.[6] The fat and lipoid content, which is probably variable, is given as about 11 per cent to 14 per cent of the dry weight. The phosphorus content of cow's spleen varies with age, the spleen of fetuses being richer in phosphorus (up to 2.4 per cent of the dry weight) while adult spleens have less phosphorus (1.3 to 1.4 per cent of the dry weight).

The sulphur content of the cow's spleen is fairly constant in the different periods of life,[7] ranging from 1.8 per cent to 2.2 per cent

[1] This work has been aided by a grant from the Rockefeller Institute for Medical Research.

[2] A portion of this work was done in the Physiological Laboratory of the University of Illinois.

[3] Oppenheimer's *Handbuch der Biochemie*, ii, 2, p. 172, 1909.

[4] S. Fränkel: *Descriptive Biochemie*, Wiesbaden, 1907.

[5] Schulz: *Pflüger's Archiv*, liv, pp. 555-573, 1893.

[6] Oppenheimer's *Handbuch der Biochemie*, ii, 2. p. 172, 1909.

[7] *Zeitschr. f. Biol.*, xxxi, pp. 400-413, 1895.

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of the dry weight. Schulz[8] examined the spleen of a man thirty-nine years old and found a water content of 79.5 per cent and a sulphur content of 0.78 per cent of the dry weight.

The iron content is also variable, differing markedly in different periods of life,[9] and the iron is held mostly in organic molecules.[10]

The proteins of the spleen have been very little studied, investigation along this line being mainly confined to the study of the nucleoproteins and purines. Mandel and Levene[11] hydrolyzed the spleen nucleoprotein and obtained glutamic acid, glycocoll, alanine, aspartic acid, proline, phenylalanine, tyrosine, lysine, arginine, histidine, adenine, guanine, cytosine and thymine.

The ammonia content of the organs of the body varies, being greater than normal during hunger, as determined by the Folin method. Grafe[12] examined one spleen, that of a horse and found 9.5 mg. of ammonia per 100 grams of spleen. The fresh spleen contains neither albumoses nor peptones. Levene[13] found as end products of the autolysis of the spleen, alanine, leucine, aminovalerianic acid, aminobutyric acid, and α-pyrrolidine carboxylic acid, phenylalanine, aspartic acid and tyrosine. Adenine and guanine were replaced by hypoxanthine and xanthine, and thymine, cytosine and uracil, which are present in spleen nucleic acid, were found as thymine and uracil after autolysis of the spleen. Jones[14] obtained from autolyzed pig spleen guanine and hypoxanthine, but no adenine, while in place of the thymine and cytosine which is found after hydrolysis, he obtained uracil. Later Jones[15] found that these differences between the purines obtained after autolysis in different experiments were due to the fact that spleens from different animals were being studied; and he determined the presence of guanase in large amounts in the cow's spleen, although this ferment was entirely absent from the pig's spleen.

[8] Schulz: *loc. cit.*

[9] Oppenheimer's *Handbuch der Biochemie*, ii, 2, p. 172, 1909.

[10] Capezuolli: *Zeitschr. f. physiol. Chem.*, lx, pp. 10-14, 1909. Burow: *Biochem. Zeitschr.*, xxv, p. 165, 1910.

[11] Mandel and Levene: This *Journal*, iii, p. xxiii, 1907-08.

[12] *Zeitschr. f. physiol. Chem.*, xlviii, p. 300, 1906.

[13] Levene: *Amer. Journ. of Physiol.*, xi, p. 437, 1904; xii, p. 275, 1904-05.

[14] Jones: *Zeitschr. f. physiol. Chem.*, xlii, p. 35, 1904.

[15] Jones: *Ibid.*, xlv, p. 84, 1905.

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Schumm[16] autolyzed spleens from cases of myelogenous splenic leucemia and obtained free guanine, xanthine, and hypoxanthine.

Schittenhelm,[17] as a result of his investigations, states that the spleen of the cow contains a hydrolytic ferment which changes adenine of hypoxanthine, and guanine to xanthine, and an oxydase which forms xanthine from hypoxanthine but does not destroy uric acid. He analyzed one sterile dog spleen, which had been autolyzed in the ice chest and then kept in alcohol for one year; it yielded on hydrolysis xanthine and hypoxanthine but no adenine nor guanine. Wells and Corper[18] have previously made note of the fact to be reported in this paper, that the dog spleen contains no uricolytic ferment, and observed that the human spleen contains no xanthine oxidase. Batelli and Stern,[19] using their method of determining uricolysis by gaseous exchange, were also unable to demonstrate uricase in the dog spleen. Jones and Austrian[20] found that normal dog spleen contained guanase, adenase and xanthineoxydase and was to this extent similar to cow spleen. Burian and Schur[21] obtained 0.16 gram of purine N from 100 grams (moist weight) of calf spleen, which was divided into 0.046 gram of free purine nitrogen and 0.101 gram of combined purine base nitrogen. Kossel[22] found in the horse spleen 0.175 per cent purine nitrogen. Jones and Winternitz[23] observed that upon autolysis of the swine spleen there was a conversion of hypoxanthine into xanthine in the absence of air. Pohl[24] states that the normal spleen of starving dogs contains no allantoin, but that after autolysis allantoin appears. He does not, however, give his experiments with autolyzed spleen. This does not agree well with the absence of uricase noted by other authors.

[16] Schumm: *Hofmeister's Beiträge*, vii, p. 175, 1905.
[17] Schittenheim: *Zeitschr. f. physiol. Chem.*, xlv, p. 84, 1905.
[18] Wells and Corper: This *Journal*, vi, p. 321, 1909.
[19] Batelli and Stern: *Biochem. Zeitschr.*, xix, p. 219, 1909.
[20] Jones and Austrian: *Zeitschr. f. physiol. Chem.*, xlviii, p. 110, 1906.
[21] Burian and Schur: *Pflüger's Archiv*, lxxx, p. 309, 1900.
[22] *Zeitschr. f. physiol. Chem.*, vi, p. 422, 1882.
[23] Jones and Winternitz: *Ibid.*, xliv, p. 1, 1905.
[24] Pohl: *Arch. f. exp. Path. u. Pharm.*, xlviii, p. 367, 1902.

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EXPERIMENTAL DATA.

Analysis of Dog's Spleen.

METHODS.—The tissues were preserved for analysis in the ice chest in ten parts or more by weight of 95 per cent alcohol, samples for water content having been taken, and when ready for analysis the alcohol was filtered off to be mixed with the rest of the alcohol and ether extracts. The total ether extract was examined quantitatively for cholesterol by Ritter's method[25] and for lecithin by the method suggested by Koch and Woods.[26] The combined residues after ether extraction were then pulverized and extracted in a shaking machine with N free water, containing alternately traces of alkali (sodium carbonate) and acid (acetic), and after bringing to faint acidity the combined watery extracts were concentrated to 1 liter and filtered hot, thus constituting the water-soluble fraction. This fraction was analyzed for purine content by the method of Krüger and Salomon,[27] and was also analyzed for a tannic acid precipitable fraction and a fraction not precipitable by tannic acid; and its phosphorus content was determined by the Neumann method as described by Koch and Woods. The remaining residue, after removal of ether and water extracts, was analyzed for its total nitrogen content by the Kjeldahl method, and for iron, phosphorus and sulphur by the method described by Koch and Mann.[28] Purine nitrogen was also determined on this residue after hydrolyzing, by means of 5 per cent sulphuric acid, using the Krüger and Salomon method. The Hausmann fractions were also determined on this tissue residue according to the modification described by Osborne and Harris.[29]

NORMAL SPLEEN A. *Results of Analyses.*—Three small spleens weighing 19.25, 31.5 and 21.5 grams, with a moisture content of 76.82 per cent, 76.50 per cent and 76.64 per cent respectively, making a total dry weight of 16.87 grams, were taken. Total ether soluble material weighed 2.609 grams, or

[25] *Zeitschr. f. physiol. Chem.*, xxxiv, p. 461, 1903.

[26] Koch and Woods: This *Journal*, i, p. 203, 1906.

[27] Hoppe-Seyler-Thierfelder: *Handbuch d. physiol. u. pathol. chem. Analyse*, 8th edition, p. 188, 1909.

[28] Koch and Mann: *Archives of Neurol. and Psychiatry*, iv. p, 20, 1909.

[29] Osborne and Harris: *Journ. of the Amer. Chem. Soc.*, xxv, pp. 323-325, 1903.

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15.47 per cent. Absolute alcohol insoluble part of this amounted to 0.2783 gram, containing 0.00082 gram of phosphorus after Neumann oxidation. The cholesterol determination was lost in this analysis.

The lecithin phosphorus found in 1 gram dry weight tissue was 0.00269 gram, figured as lecithin=0.0694 gram.

Non-lecithin phosphorus in this fraction = 0.0005 gram in 1 gram dry tissue.

Water Soluble Fraction:

	gram
Nitrogen precipitable by tannic acid in 1 gram dry tissue	=0.00567
Nitrogen not precipitable by tannic acid in 1 gram dry tissue	=0.00396
Water soluble N in 1 gram, total	=0.00972
Water soluble phosphorus in 1 gram dry tissue	=0.0052

Purines found in water soluble fraction, only a doubtful trace.

Tissue Residue (Insoluble) Fraction. One gram of tissue (after extraction of soluble constituents) yielded 0.0035 gram of iron, 0.0070 gram of sulphur, and about 0.0084 gram of phosphorus.

One gram of dry tissue residue (used 8.4 grams for analyses) yielded 0.00345 gram of purine nitrogen.

Total nitrogen determination (using 0.28 gram tissue residue) yielded 0.1447 gram of nitrogen in one gram of tissue residue.

NORMAL SPLEEN B. One large spleen with a moist weight of 134 grams, and a moisture content of 75.59 per cent, making a total dry weight of 32.71 grams was taken.

Total ether-soluble material weighed 3.8155 grams or 11.65 per cent.

Absolute alcohol insoluble part of this = 0.6861 gram, containing 0.0140 gram of phosphorus (after Neumann oxidation.)

One gram dry tissue contained 0.015 cholesterol by the Ritter method (using one-half of the total ether extract for the analysis).

The lecithin fractions were lost in this analysis.

Water Soluble Fraction:

Nitrogen precipitated by tannic acid in 1 gram dry tissue	=0.00412
Nitrogen not precipitated by tannic acid in 1 gram dry tissue	=0.00393
Water soluble N in 1 gram dry tissue, total	= 0.00804
Water soluble phosphorus in 1 gram dry tissue	= 0.00346

Purines found in water soluble fraction = a doubtful trace.

Tissue Residue (Insoluble) Fraction. One gram of tissue residue (freed from soluble constituents) yielded 0.00455 gram of iron, 0.00714 gram of sulphur, and 0.00459 gram of phosphorus.

One gram of dry tissue residue (used duplicates of about 6 grams each) yielded 0.00292 gram of purine nitrogen.

Total nitrogen determination (using about 0.25 gram tissue residue in duplicates) yielded 0.1639 gram of nitrogen in one gram of tissue residue.

NORMAL SPLEEN C. One large spleen with a moist weight of 82 grams and a moisture content of 76.94 per cent making a total dry weight of 18.91 grams.

Total ether soluble material weighed 2.859 grams or 15.11 per cent.

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Absolute alcohol insoluble part of this = 0.2021 gram, containing 0.00339 gram of phosphorus (after Neumann oxidation).

Cholesterol determination was far too low, due to loss occasioned by the Ritter method, which will be discussed in a future paper.

The lecithin phosphorus found in 1 gram dry tissue = 0.00241 gram, figured as lecithin = 0.0622 gram.

Non-lecithin phosphorus in this fraction = 0.00015 gram in 1 gram dry tissue.

Water Soluble Fraction:

Nitrogen precipitated by tannic acid in 1 gram dry tissue = 0.0012
Nitrogen not precipitated by tannic acid in 1 gram dry tissue = 0.00338

Total water soluble N in 1 gram dry tissue..... = 0.00458
Water soluble phosphorus in 1 gram dry tissue..... = 0.0027

Only doubtful trace of purines was found in the water soluble extractives.

Tissue Residue (Insoluble) Fraction.—One gram of tissue residue yielded 0.0122 gram of iron, 0.00745 gram of sulphur, and 0.00489 gram of phosphorus.

One gram of dry tissue residue (used about a 6 gram sample) yielded 0.00428 gram of purine nitrogen.

Total nitrogen determination (using about 0.26 gram, tissue residue in duplicates) yielded 0.1629 gram of nitrogen in 1 gram of tissue residue.

Hausmann Fractions on tissue residue C.—Duplicate analyses were obtained with the following result-, from 1 gram of dry t ssue residue:

Amid N	=0.01349	and	0.01329	gram	N — mean	=0.01339
Humus N	=0.00938	and	0.00751	gram	N — mean	=0.00844
Diamino N	=0.03546	and	0.03244	gram	N — mean	=0.03395
Monamino N	=0.1007	and	0.0969	gram	N — mean	=0.09880
					Total N	=0.15458

Purines and Purine Enzymes in Dog Spleen.

I. Hydrolysis of Dog Spleen.—1091 grams of dog spleen (moist weight) were hydrolyzed by means of 5 per cent sulphuric acid and the purines isolated in the pure state and weighed, with the following results, figured on the basis of 1 gram moist weight of the original spleen tissue used:

Guanine	=0.00109 gram (weighed as such).
Adenine	=0.00062 gram (weighed as pierate).
Hypoxanthine	=0.00015 gram (weighed as silver nitrate combination).
Xanthine	=0.00004 gram (weighed as such).

Total products =0.00190 gram.

No uric acid was found, although tested for.

A purine nitrogen figure was obtained (using one-eightieth of the total material) and yielded in the figures of 1 gram moist weight of the original spleen tissue, 0.00126 gram of nitrogen.

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Nitrogen figure obtained from the total amount of isolated purines was 0.000899 gram per 1 gram moist splenic tissue.

II. Autolysis of Dog Spleen in the Absence of Air.—552 grams of dog spleen (moist weight) were autolyzed for about a month, part of the time at 37° C. and using toluene as antiseptic and to keep out air. The autolysate was examined quantitatively for liberated purines and gave the following results, on the basis of 1 gram moist weight of original spleen tissue used:

No guanine, adenine or uric acid was found.

The major portion of the purine product was

Xanthine	=0.00168 (weighed as such, and free from uric acid).
Hypoxanthine	=0.00002 gram (weighed as silver nitrate salt).
Total products	=0.00171 gram.

Calculated purine nitrogen in these products = 0.00063 gram N, indicating that about half the total purine nitrogen was present in the form of free purines.

III. Autolysis of Dog Spleen in the Presence of Air.—617 grams of dog spleen (moist weight) were autolyzed for about a month, part of which time a current of air was passed through the autolyzing mixture, and the temperature frequently brought to 37° C., toluene being used as antiseptic. The autolysate was examined quantitatively for purines, with the following results, on the basis of 1 gram moist weight of original spleen tissue used:

Neither guanine or adenine were found.

The major portion of the purines was

Uric acid	=0.001695 gram (weighed as such. Repurified from concentrated H_2SO_4).
Xanthine	=0.000094 gram (weighed as such).
Hypoxanthine	=0.000004 gram (weighed as hypoxanthine silver nitrate).
Total products	=0.001793 gram

Calculated purine nitrogen in these products =0.0006 gram or about half the total purine nitrogen.

IV. Xanthine-Oxidase of Dog Spleen.—Fifty grams of finely ground fresh dog spleen, mixed with three volumes of toluene water, was allowed to stand at room temperature over night, and strained through cheese cloth the following morning. To this spleen extract thus obtained was added 0.1355 gram of xanthine in solution, and the mixture was placed in a bottle with sufficient toluene to prevent putrefaction, and connected with another bottle containing toluene and water, through which was drawn the air that then passed through the digestion mixture, and all the bottles were kept at about 40° C. for twenty-four hours. After autolysis 0.0969 gram of uric acid was recovered, which gave a positive murexide test, and upon repurification from H_2SO_4, pure uric acid was obtained, thus differentiating it from xanthine.

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V. Uricase of Dog Spleen. (*Experiment 1.*)—Sixty-three grams of ground up dog spleen, and 50 grams of ground up dog's liver as control (high uricolytic power of dog liver having been demonstrated) were each mixed with three volumes of saturated toluene water and strained through cheese cloth after standing over night at room temperature. To the spleen extract thus obtained was added a solution of 0.1735 gram of uric acid, and to the liver extract was added 0.1616 gram of uric acid. Both were then kept at about 40° C. with a supply of air passing through them, for 48 hours and they were then analyzed for uric acid. From the spleen extract was recovered 0.1919 gram of uric acid, while the liver extract did not contain a trace of uric acid.

Experiment 2.—Eighty grams of ground up dog spleen were allowed to stand at room temperature over night, and was then strained through cheese cloth. The extract was divided into two equal portions: to No. I was added 0.1655 gram uric acid in solution, and to No. II, after boiling fifteen minutes was added 0.1962 gram uric acid in solution. Both were then kept at about 40° C. for twenty-four hours, during which time they received a supply of air bubbling through them and were then analyzed for uric acid content. From No. I was recovered 0.1687 gram uric acid (which when recrystallized from H_2SO_4 yielded 0.1638 gram) and from No. II which had been boiled, was recovered 0.1856 gram of uric acid.

SUMMARY.

1. Average of analyses of three normal dogs' spleens resulted as follows:

A moisture content of about 75 to 77 per cent.

A content of ether-soluble materials between 11.6 and 15.5 per cent of the dry weight, which was made up of about 1.5 per cent. cholesterol and between 6 and 7 per cent lecithin (leaving but 2 to 6.5 per cent neutral fats.)

The total soluble nitrogen ranged between 0.45 per cent and 0.97 per cent of the dry weight, divided about equally between that precipitable and that not precipitable with tannic acid.

A water-soluble phosphorus content of about 0.27 to 0.52 per cent.

No purines were found in the water-soluble fraction, at least not in sufficient quantity from the amounts of tissue used to be recognized as such.

The insoluble part of the tissue contained about 0.26 to 0.98 per cent of dry weight as iron, 0.53 to 0.60 per cent of dry weight as sulphur, and about 0.39 per cent of dry weight as phosphorus,

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with a purine nitrogen content of 0.24 to 0.35 per cent of the dry weight.

The total nitrogen content of the insoluble part was about 11.0 to 13.4 per cent of the dry weight, which was distributed as follows: Amid N, 8.60 per cent; Humus N, 5.76 per cent; Diamino N, 21.87 per cent; and Monamino N, 63.71 per cent.

In contrast to normal liver, examined by Wells[30] by the same method, the insoluble residue of the spleen contained a slightly larger percentage of its dry weight as iron, about the same percentage of sulphur and a greater percentage of phosphorus. The Hausmann nitrogen fractions were also slightly different; more amid nitrogen, about the same or a little more humus nitrogen, less diamino nitrogen and about the same percentage of monamino nitrogen.

2. The purines obtained from 1 kilo (moist weight) of dog spleen after hydrolysis were: Guanine, 1.09 gram, adenine, 0.62 gram; hypoxanthine, 0.15 gram, and xanthine 0.04 gram. Analyzed purine nitrogen content in one kilo moist weight = 1.62 grams.

3. The purines obtained from the autolysate from 1 kilo (moist weight) dog spleen, after autolysis in the absence of air, was 1.69 grams of xanthine and 0.017 gram of hypoxanthine.

4. The purines obtained from the autolysate from 1 kilo (moist weight) of dog spleen, upon autolysis in the presence of air, were 1.69 grams of uric acid, 0.09 gram of xanthine and 0.004 gram of hypoxanthine.

5. Of the purine enzymes, evidence was obtained of the presence of xanthine oxidase, adenase and guanase, while uricase was lacking. The conversion of hypoxanthine to xanthine during comparative anaerobic conditions indicates the presence of an oxidizing enzyme with this particular function; whether it be the xanthine oxidase itself, or a special hypoxanthine oxidase.

[30] Wells: This *Journal*, v, pp. 141-142, 1908.

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ERRORS IN THE QUANTITATIVE DETERMINATION OF CHOLESTEROL BY RITTER'S METHOD: THE INFLUENCE OF AUTOLYSIS UPON CHOLESTEROL.[1]

BY HARRY J. CORPER.

(*From the Pathological Laboratory of the University of Chicago.*)[2]

(Received for publication, December 3, 1911.)

While carrying out analyses of normal and autolyzed spleens reported in previous papers[3] the Ritter method[4] for determining cholesterol quantitatively was found inadequate. The results were so variable for the amounts used that it was thought advisable to investigate the steps in the method in order to find the source of error, and also to find out whether the method actually could be used for the quantitative determination of cholesterol in tissues. At least one other investigator, Helen Baldwin,[5] has had difficulty in the quantitative determination of cholesterol by this method.

The quantitative methods for cholesterol determination suggested up to 1908 (a discussion of these methods is given by Glikin[6]) practically all depend either upon the saponification of the fat to be examined, the cholesterol being recovered as such, or upon the esterification of the cholesterol and the determination of the iodine figure or saponification number.

More recently Windaus[7] has suggested a new method for the determination of cholesterol and cholesterol esters, precipitating

[1] This work has been aided by a grant from the Rockefeller Institute for Medical Research.

[2] A portion of this work was done in the Physiological Laboratory of the University of Illinois.

[3] Cf. preceding article on The Chemistry of the Dog's Spleen and reference 23, page 44.

[4] E. Ritter: *Zeitschr. f. physiol. Chem.*, xxxiv, p. 461, 1903.

[5] Helen Baldwin: This *Journal*, iv, p. 213-219 (218), 1908.

[6] W. Gilkin: *Biochem. Centralbl.*, vii, pp. 289-306; 357-77, 1908.

[7] A. Windaus: *Zeitschr. f. physiol. Chem.*, lxviii, pp. 110-117, 1910.

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them by means of digitonin. Lapworth[8] reports good results using this method.

In order to more clearly understand where loss in Ritter's method might occur, the literature was searched for studies on the action of the various chemicals used in the method upon cholesterol in forming salts, or actual decomposition products and the properties of these compounds.

Lindenmeyer[9] prepared sodium cholesterolate, which he purified from chloroform by cooling the solution on ice, and found it to be insoluble in water, and that it was only slowly decomposed by water, weak alcohol hastening this decomposition. Obermüller[10] prepared potassium cholesterolate and found the properties similar to those of the sodium compound. Darmstädter and Lifschutz[11] were able to obtain oxidation products of cholesterol by heating cholesterol with alcoholic potash with a reflux condenser for eight hours, the yield being 20 to 25 per cent. Lifschutz[12] divided the products into three phases: (1) Oxycholesterin ethers; (2) Oxycholesterins and (3) Dicarbonsäure (Chollansäure), the first two being soluble in all the ordinary solvents except water, and the last being soluble in water and alkalies and precipitating as white flocks on acidification. Schulze and Winterstein[13] noted a drop in the melting point of cholesterol exposed to the light, apparently due to oxidation, as an atmosphere of CO_2 prevented this change.

Lifschutz[14] states that one hour's cooking with half-normal alcoholic potash does not alter cholesterol. E. Schulze[15] recovered cholesterol from substances in which potash did not liberate it so that it could be extracted by ether, by heating with benzoic acid in sealed tubes and forming an ester insoluble in alcohol and ether.

[8] A. Lapworth: *Journ. of Path. and Bact.*, xv, pp. 254-61, 1911.

[9] Lindenmeyer: *Erdmann's Praktische Chemie*, xc, pp. 321-332, 1863.

[10] Obermüller: *Zeithschr. f. physiol. Chem.*, xv, pp. 37-48, 1891.

[11] L. Darmstädter and J. Lifschutz: *Ber. d. deutsch. chem. Ges.*, xxxi, pp. 1122-27, 1898.

[12] J. Lifschutz: *Zeitschr. f. physiol. Chem.*, l, pp. 436-40, 1906-07.

[13] E. Schultz and E. Winterstein: *Ibid.*, xliii, pp. 316-19, 1905; xlviii, pp. 546-48, 1906,

[14] J. Lifschutz: *Ibid.*, lviii, p. 175, 1908.

[15] E. Schulze: *Zeitschr. f. anal. Chemie*, xvii, p. 173.

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Very little reliable work has been done on the changes occurring in cholesterol during autolysis, probably in part because of the fact that no reliable quantitative cholesterol method has been available, and partly because the change occurring, if such does occur, is naturally a slow one. Windaus,[13] method for determining cholesterol and cholesterol esters, and Lifschutz's[17] recent investigations upon the determination of the presence of oxidation products of cholesterol, should make research fruitful along these lines.

Moore[18] failed to find any change in the cholesterol content of the liver in autolysis under toluene for forty-two days at 37°C. (Cholesterol analyzing 0.038 per cent before and 0.0372 per cent after autolysis). He also found no significant difference between the cholesterol content of a normal area (0.64 per cent) and an infarcted area (0.58 per cent) in a human spleen, and strongly objects to the reasoning of Carbone,[19] who believes that cholesterol originates from lecithin by decomposition, and of Waldvogel[20] who claims to have established the same by digesting lecithin with sterile liver juice, and who also found an increased cholesterol content in pathological livers as compared to normal (normal cholesterol content being 0.42 per cent, pathological—acute poisoning—being 24.46 per cent according to these analyses.)

EXPERIMENTAL PART.

METHODS. Ritter puts 50 grams of fat into a porcelain dish, adds 100 cc. of alcohol, brings it to a boil on the water bath, and then adds 8 grams of sodium dissolved in 160 cc. of 99 per cent alcohol with constant stirring. (The sodium alcoholate is prepared according to the method described by Kossel and Krüger.[21] These authors bring the absolute alcohol to a boil under a reflux condensor and carefully add the metallic sodium to it while boiling. They state that 10 cc. of a 5 per cent sodium alcoholate

[16] Windaus: *loc. cit.*
[17] J. Lifschutz: *Zeitschr. f. physiol. Chem.*, liii, pp. 140-48, 1907.
[18] F. Craven Moore: *Medical Chronicle*, xlvii, pp. 204-40, 1907-08.
[19] Tito Carbone: *Arch. ital. de biol.*, xxvi, p. 279, 1896.
[20] Waldvogel and Mette: *Münch. med. Woch.*, liii, p. 402, 1906.
[21] A. Kossel and M. Krüger: *Zeitschr. f. physiol. Chem.*, xv, p. 321, 1891.

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solution thus prepared will saponify 5 grams of mutton tallow, and 15 cc. will saponify 5 grams of butter fat).[22] The alcohol is then evaporated off on the water bath and about one and one-half times as much salt as fat used is added, and enough water so that most of the contents of the evaporating dish goes into solution. This is then dried on the water bath with constant stirring, and then at 80°C, in a drying oven. It is pulverized, put into a sulphuric acid dessicator for a short time, then into an extraction thimble, and is extracted in a Soxhlet apparatus with ordinary ether for nine hours. The ether extract is then put into a separatory funnel and shaken out with water to remove glycerin. The ether extract is dried, dissolved in hot alcohol, precipitated by means of water, precipitate dried at 100-120°C. and weighed.

Experiments on the Effect of Sodium Alcoholate upon the Quantitative Yield of Cholesterol by Ritter's Method.

In order to test the loss occasioned by the steps in the method, the following experiments were carried out, using pure cholesterol instead of a complex fat mixture.

EXPERIMENT 1. The amount of cholesterol which can be recovered from salt mixture, being mixed in alcohol solution, dried, and extracted by means of absolute ether in a Soxhlet apparatus, and the ether extract shaken out by means of water.

(a) Used 0.1016 gram cholesterol and recovered 0.1026 gram.

(b) Used 0.1020 gram cholesterol and recovered 0.1030 gram.

EXPERIMENT 2. Amount of cholesterol which can be recovered from salt mixture after treating with sodium alcoholate, evaporating to dryness, dissolving residue in ether and shaken out by means of water.

(a) Used 0.1057 gram cholesterol mixed with 5 cc. of 5 per cent sodium alcoholate and recovered 0.1048 gram cholesterol.

(b) Used 0.1007 gram cholesterol mixed with 10 cc. of 5 per cent sodium alcoholate and recovered 0.1016 gram cholesterol.

(c) Used 0.1005 gram cholesterol mixed with 40 cc. of sodium alcoholate and recovered 0.0950 gram cholesterol.

(d) Duplicate of (c). Used 0.1003 gram cholesterol and recovered 0.1020 gram cholesterol.

EXPERIMENT 3. Amount of cholesterol which can be recovered after solution in absolute alcohol, saponification by means of 10 cc. of sodium

[22] The sodium alcoholate employed in the following experiments was prepared according to this method, 5 per cent strength being used.

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alcoholate, evaporated to dryness, mixing with salt (10 to 15 grams), and extraction by means of absolute ether in a Soxhlet for 9 hours, shaking out ether extract with water, etc.

(a) Used 0.1013 gram cholesterol and recovered 0.0390 gram.

(b) Used 0.1012 gram cholesterol and recovered 0.0437 gram.

EXPERIMENT 4. Similar to Experiment 3 but using only 5 cc. of sodium alcoholate.

(a) Used 0.1016 gram cholesterol and recovered 0.0847 gram.

(b) Used 0.1042 gram cholesterol and recovered 0.0858 gram.

EXPERIMENT 5. Similar to Experiment 3 but using 40 cc. of sodium alcoholate.

(a) Poured on salt in absolute alcohol solution, after saponifying. Used 0.1014 gram cholesterol and recovered 0.0415 gram.

(b) Duplicate of (a). Used 0.1016 gram cholesterol and recovered 0.0390 gram.

(c) Evaporated to dryness after saponification and emulsionized by means of water, mixed with salt, dried, extracted with ordinary ether, etc.

Used 0.1032 gram cholesterol and recovered none.

(d) Duplicate of (c). Used 0.1046 gram cholesterol and recovered *none*.

(e and f) Not evaporated to dryness after saponification, emulsionized with saturated salt solution, dried, ground up, extracted with absolute ether, etc.

Used 0.1023 gram cholesterol and recovered 0.0163 gram.

Used 0.1002 gram cholesterol and recovered 0.0150 gram.

EXPERIMENT 6. Amount of cholesterol that can be recovered after saponification by means of 40 cc. of sodium alcoholate, evaporating as nearly dry as possible, dissolved in ether and in water and these extracts poured on salt and dried and then extracted in a Soxhlet by means of absolute ether, etc.

(a) Used 0.1010 gram cholesterol and recovered 0.0770 gram.

(b) Used 0.1003 gram cholesterol and recovered 0.0700 gram.

As a result of the above experiments we can conclude that even an excess of 5 cc. of 5 per cent sodium alcoholate added to cholesterol, will prevent its complete extraction from a dried salt mixture by means of ether.

That the trouble lies in the use of an excess of sodium alcoholate is further shown by the following experiments:

In order to simplify matters the following abbreviation is used for the various steps.

A. Cholesterol dissolved in absolute alcohol and heated on water bath for three days.

B. Saponifying with sodium alcoholate.

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C. Mixing with NaCl and drying.
D. Extracting in a Soxhlet with absolute ether.
E. Shaking out the ether extract with water in a separatory funnel.
F. Allowing the ether to evaporate off at room temperature, and drying the residue at 100° C and weighing.

TABLE OF RESULTS.

Cholesterol.

COMBINATIONS	USED	RECOVERED	LOSS
	gram	*gram*	*gram*
A. F	0.1068	0.1075	0.00
E. F	0.1001	0.1013	0.00
A. C. D. E. F	0.1001	0.0969	0.0032
B. C. D. E. F	0.1096	0.0955	0.0141
A. B. C. D. E. F	0.1061	0.0675	0.0386

In the above experiments the exact amount of sodium alcoholate used was not noted, as they were carried out before the first set cited.

Now if an excess of sodium alcoholate thus affected the yield of cholesterol by the Ritter method when pure cholesterol was used, what would be its effect upon the cholesterol yield from tissues? In this case we are unable to tell the exact amount of fats and esters present, and therefore the amount of sodium alcoholate necessary to saponify them. If we use too small an amount our result will be high, due to the unsaponified fats and esters remaining as such with the cholesterol; and if we use too much the cholesterol yield will be low. To test these points the following experiments were carried out in the ether and alcohol extract from a steer spleen.

The steer spleen weighed about 850 grams (moist) and yielded an alcohol and ether extract weighing 29.13 grams, which was dissolved in a liter of absolute alcohol and divided in 50 cc. samples (5 per cent. of the total extract) for the following analyses for cholesterol.

Cholesterol.

EXPERIMENT 1. The extract after evaporation was dissolved in 10 cc. of absolute alcohol and warmed, 10 cc. of 5 per cent sodium alcoholate was added and the mixture warmed several hours, evaporated to dryness, redissolved in absolute alcohol, poured on 10 to 15 grams of salt, dried, extracted in a Soxhlet with absolute ether.

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Results. (a) Yielded 0.0263 gram cholesterol.
(b) Yielded 0.0195 gram cholesterol.

EXPERIMENT 2. Identical with Experiment 1 except that the saponified mixture was poured directly on the salt (without evaporation).

Results. (a) Yielded 0.0162 gram cholesterol.
(b) Yielded 0.0380 gram cholesterol.

EXPERIMENT 3. Identical with Experiment 2 except 30 cc. of absolute alcohol was used as a solvent before saponification.

Results. (a) Yielded 0.0215 gram cholesterol.
(b) Yielded 0.0528 gram cholesterol.

EXPERIMENT 4. Identical with Experiment 2, but used 40 cc. of sodium alcoholate and evaporated the saponified mixture as nearly to dryness as possible, redissolved and poured on salt.

Results. (a) Yielded 0.0072 gram cholesterol.
(b) Yielded 0.0066 gram cholesterol.
(c) and (d) were not evaporated after saponification before adding to the salt.
(c) Yielded 0.0050 gram cholesterol.
(d) Yielded 0.0100 gram cholesterol.

EXPERIMENT 5. Identical with Experiment 1 except that only 5 cc. of sodium alcoholate was used.

Results. (a) Yielded 0.1773 gram cholesterol.
(b) Yielded 0.1600 gram cholesterol.

EXPERIMENT 6. The object of this experiment was to compare an old preparation of sodium alcoholate (five months old) and of dark brown color with the freshly prepared compound. It is practically a duplicate of Experiment 5 but using 5 cc. of old sodium alcoholate.

Results. (a) Yielded 0.1624 gram cholesterol.
(b) Yielded 0.1840 gram cholesterol.

EXPERIMENT 7. Resembled Experiment 5 except in that the mixture was not evaporated to dryness after saponification but poured directly on the salt after standing 24 hours.

Results. (a) Yielded 0.1306 gram cholesterol.
(b) Yielded 0.1312 gram cholesterol.

EXPERIMENT 8. Identical with Experiment 1 but used 3 cc. 5 per cent sodium alcoholate. (The resulting product was only slightly oily in appearance).

Results. (a) Yielded 0.2180 gram cholesterol.
(b) Yielded 0.2155 gram cholesterol.

EXPERIMENT 9. Identical with Experiment 8, but used only 1 cc. of sodium alcoholate. (The product was not crystalline but oily).

Results. (a) Yielded 0.3543 gram cholesterol.
(b) Yielded 0.2956 gram cholesterol.

As a result of the above experiments we can conclude that the best yield of cholesterol is obtained from the alcohol and ether

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extract of the spleen when about 5 cc. of 5 per cent sodium alcoholate is used to saponify 1.5 gram of the ether-aclohol extract; we must, however, expect an error in the quantitative results on this amount of extract of from 10 per cent to 25 per cent.

Cholesterol in Autolysis.

Remembering the possibility for analytical error by the Ritter method as shown above, the following experiments can carry no great weight, but will merely be cited to show that there is no marked change in the cholesterol content of the spleen (dog) during autolysis.

EXPERIMENT 1. Sixty-five grams of ground dog spleen were mixed with 0.338 gram of cholesterol suspension (made by dissolving the cholesterol in a minimum amount of absolute alcohol and pouring it into 0.9 per cent sodium chloride solution), toluene was used as preservative and the mixture allowed to autolyze for ninety-two hours at room temperature.

Recovered 0.591 gram of cholesterol or 0.253 gram above the amount added which must have come from the spleen. (Extraction, etc., was carried on here as in the case of the steer spleen analyses.)

EXPERIMENT 2. Sixty-five grams of ground up dog spleen was mixed with 0.314 gram cholesterol (suspended in 0.9 per cent NaCl-toluene water). The mixture autolyzed at room temperature for fifty-three hours.

Recovered 0.5566 gram cholesterol or 0.242 gram of cholesterol over the amount added.

As a few successful analyses for cholesterol were obtained while carrying out the autolysis experiments reported in previous papers[23] they may be put into tabulated form for comparison with the two above mentioned experiments. For the sake of convenience the figures will be given in the form of the amount of cholesterol found in one gram dry weight of spleen (on a basis of 23 per cent of solids in fresh spleen). In Experiments 1 and 2 above only the cholesterol content of the splenic tissue is given (that obtained by deducting the cholesterol added).

[23] Work to be published in the *Journal of Experimental Medicine* upon correlation of chemical and histological changes.

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SPLEEN	TIME AUTOLYZED	CHOLESTEROL PER 1 GRAM DRY WEIGHT SPLEEN
		gram
Normal (Spleen B. Ref. 3)	0.00	0.0150
Experiment 2 (above)	53 hours	0.0162
Experiment 1 (above)	92 hours	0.0169
*Six days autolysis (spleen H, Ref. 3)	6 days	0.0216
*Two days *invivo* autolysis (Spleen I, Ref. 2)	48 hours *in vivo*	0.0217

*These two spleens were analysed at the same time; the normal spleens above being analysed at an earlier period.

The last two cholesterol figures though differing from the first three by about 20 per cent are still within the error limit of the method.

In conclusion we can say then that within the limit of error of the Ritter method for cholesterol, this constituent of the tissues does not markedly change in amount during autolysis.

GENERAL SUMMARY.

1. A source of error was found in the quantitative estimation of cholesterol by the Ritter method, in the fact that the presence of an excess of sodium alcoholate over that necessary for the saponification of the fats and esters, prevents a complete extraction of the cholesterol from the salt mixture by means of ether.

2. This error may vary from 5 per cent to 20 per cent in the case of a normal tissue when there is an excess of from 1 cc. to 3 cc. of a 5 per cent sodium alcoholate solution used in the saponification of 1.5 grams of the alcohol-ether extract.

3. The Ritter method for the quantitative determination of cholesterol in tissues should be used only with certain restrictions and precautions in mind.

4. No marked change was found in the amount of cholesterol present in the dog spleen after *in vitro* and *in vivo* autolysis of short duration.

5. The steer spleen contains about 0.4 per cent of its moist weight as cholesterol.

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HEREDITY OF PIGMENTATION IN FUNDULUS HYBRIDS

FRANK W. BANCROFT

From the Department of Experimental Biology, Rockefeller Institute, New York

THIRTY FIGURES

Ever since the rediscovery of Mendel's law, students of heredity who have investigated characters in the adult have habitually sought to determine whether or not these characters were inherited according to the Mendelian formula. Those, however, who have investigated the inheritance of larval and embryonal characters have usually not considered the facts from the Mendelian point of view but have sought to determine whether the inheritance was maternal or paternal. While it is possible that this point of view may be the most fruitful one from which to regard hybrids of distantly related species; still the work of Loeb, King, and Moore,[1] showed that for the larvae of closely related sea-urchins, at any rate, the Mendelian point of view could be adopted with profit. They found that in the larvae obtained by crossing Strongylocentrotus purpuratus and S. franciscanus certain characters of each species were dominant over the allelomorphic character in the other species, and the result was the same no matter whether the character in question was maternal or paternal. On account of the wealth of teleost material in Woods Hole and its favorable character for such investigations it was suggested by Dr. Loeb, whose constant helpfulness during the course of the work I wish to acknowledge, that I take up the study of inheritance in Fundulus from the Mendelian and the physiological points of view.

Previous work on Mendelian inheritance of this form is limited to the paper by Newman[2] who came to the conclusion that

[1] Arch. f. Entwick-mech. 1910, Bd. 29, p. 354.
[2] Jour. Exp. Zool., vol. 5, p. 503.

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although some characters, such as the size and shape of the chromatophores, and the color pattern on head and body, show Mendelian dominance, that "nearly all of the characters observed may be classed as examples of blended inheritance of one sort or another." [3]

METHODS

Reciprocal crosses between Fundulus heteroclitus and Fundulus majalis were made in the usual way; both eggs and sperm from a number of individuals of each species being mixed for each experiment. The developing embryos were kept in Syracuse watch-glasses and fingerbowls, loosely covered with glass. The water was frequently changed and the eggs were carefully separated from the masses to insure a maximum supply of oxygen. After hatching the young fish were usually kept isolated in fingerbowls. They were fed with what they could pick off of foul eelgrass, and small pelagic organisms obtained by towing. Later fish flesh, flies, and liver were used for food. Some of the little fish were put out in the eel-pond in cloth cages of various kinds, but though they grew much faster than any that were kept in the laboratory so many of the cages got broken that only one of these fish was kept until the middle of September when they were transferred from Woods Hole to New York.

INHERITANCE OF COLOR CHARACTERS

Three kinds of chromatophores were observed, all three of them occurring in all the embryos of both pure species, and of both kinds of hybrids. They are:

1. Black opaque chromatophores. These are the first to appear, and apparently persist throughout the life of the fish.

2. Red opaque chromatophores, usually of a brick red or deep yellow color, showing white or creamy with a closed diaphragm. They sometimes take on a white or creamy color but can usually be easily distinguished from other tissues by their conspicuous white color when the light is turned off. They are nearly as

[3] Jour. Exp. Zool., vol. 5, p. 355.

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large as the black chromatophores, appear in early embryonal life at about the same time as the black or but shortly after them, and disappear completely within a few days after hatching.

3. Small lemon yellow, or greenish yellow chromatophores. The pigment in these is transparent and it is only by carefully observing them in the most favorable locations such as the fins that the branched processes and the chromatophore-like shape of the cell can be made out. They first appear a few days before hatching and persist as long as the fish have been observed.

As all three kinds of chromatophores were found in all the embryos, and as no small differences in the color of the chromatophores that seemed to be significant were seen, the color differences observed were due to the variation of the chromatophores in (1) number, (2) size and shape, (3) location or arrangement, (4) rate of appearance and development.

Table 1 gives a summary of the main features of the pigmentation in the four forms studied.

An inspection of the table will show that in the first four characters (viz.: the red and black yolk chromatophores, head chromatophores, and red chromatophores of the lateral line) all of which are not concerned with the rate of development there is a well marked Mendelian dominance, while in the last two characters (viz.: the arrangement and time of appearance of the yolk chromatophores) both of which are concerned with the rate of development the dominance is not so evident. The characters will be considered *seriatim*.

1. Red yolk chromatophores

The number of the red yolk chromatophores of the pure F. heteroclitus and both kinds of hybrids is about the same. No attempt was made to count them accurately but in all these three forms the red yolk chromatophores are very conspicuous features. In F. majalis, on the other hand, these chromatophores are so few that in one of the series they could not be found at all. In other series the first red yolk chromatophores appeared near the embryo from the fifth to the ninth day and increased slightly in

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TABLE 1

Principal color differences between pure forms and hybrids

PURE F. HETEROCLITUS	F. HETEROCLITUS ♀ × F. MAJALIS ♂ F. HETEROCLITUS EGG HYBRID	F. MAJALIS ♀ × F. HETEROCLITUS ♂ F. MAJALIS EGG HYBRID	PURE F. MAJALIS
1. Red yolk chromatophores large and abundant.	Red yolk chromatophores large and abundant, of same shape but smaller than in previous form.	Red yolk chromatophores large and abundant, but a little smaller, and less branched than in pure F. heteroclitus.	Red yolk chromatophores much fewer also smaller and less branched than in other three forms.
2. Black yolk chromatophores large and of a general polygonal shape, processes usually wanting.	Black yolk chromatophores similar to those of previous class but a little smaller and with many minute processes.	Black yolk chromatophores similar to those of pure F. heteroclitus in early stages.	Black yolk chromatophores smaller, and with more and longer branches than in pure F. heteroclitus.
3. First crop of head chromatophores present.	First crop of head chromatophores present.	First crop of head chromatophores present.	First crop of head chromatophores absent.
4. Lateral line with a continuous line of red chromatophores at and before hatching.	Lateral line about the same as in previous class but the red chromatophores begin to fade sooner.	Lateral line about the same as in pure F. heteroclitus.	Lateral line without red chromatophores.
5. Lateral line with none or but few black chromatophores upon hatching.	Lateral line with a few more black chromatophores upon hatching than in pure F. heteroclitus.	Lateral line with comparatively few black chromatophores.	Lateral line with about 50 or 60 black chromatophores upon hatching.
6. When the yolk chromatophores first appear there are quite or nearly as many on the surface of the yolk sac opposite to the embryo as on any other part.	Yolk chromatophores at first about evenly distributed as in previous class.	Yolk chromatophores at first absent or decidedly fewer in that part of the yolk sac opposite to the embryo.	Yolk chromatophores first appear at the sides of the embryo, and are entirely absent from the hemisphere opposite to the embryo.
7. First yolk chromatophores appear earlier than in any of the other forms.	First yolk chromatophores appear later than in previous but earlier than in subsequent forms.	First yolk chromatophores appear later than in previous, but earlier than in following form.	First yolk chromatophores appear later than in any of the other forms.

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number though still remaining in the vicinity of the embryo. After a few days their number again decreased markedly, apparently by incorporation within the embryo, so that they could only be found in a small proportion of the eggs, and in these not more than two or three cells to each yolk sac. There is no doubt then, that, so far as the *number* of these cells is concerned, there is a well marked Mendelian dominance, but as it is almost impossible to count them accurately I cannot be sure that in the hybrids the number of red yolk chromatophores is not slightly less than in pure F. heteroclitus.

An examination of the red yolk chromatophores in the four forms for *size* showed that the chromatophores of F. majalis were usually much smaller than those of any of the other forms; but that these cells in the F. majalis egg hybrid were slightly smaller and in the F. heteroclitus egg hybrid considerably smaller than in the pure F. heteroclitus (compare figs. 13 and 14; 15, 16, and 17; 18 and 19; 20 and 21; 22 and 23). As in most cases where the hybrids showed a condition intermediate between those of the two pure species it was the F. heteroclitus egg hybrid which was most like the pure F. heteroclitus; this case in which the F. majalis egg hybrids were most like the pure F. heteroclitus deserves special emphasis.

In *shape* the red yolk chromatophores of both hybrids approximated closely to the dominant condition of the pure F. heteroclitus. When they first appeared, these chromatophores in all four forms had about the shape of the red yolk chromatophore in fig. 13. In the pure F. majalis the red yolk chromatophores even at the period of their maximum complexity (fig. 17) did not progress far beyond this early shape; while the chromatophores of the three other became much larger and more complex. The F. heteroclitus egg hybrids usually had red yolk chromatophores of exactly the same shape as the pure F. heteroclitus (compare figs. 18 and 19, 20 and 21, 22 and 23). The F. majalis egg hybrids also in many cases had cells of exactly the same shape and almost the same size, as those of the pure F. heteroclitus. In some cases, however, the processes of these cells in the hybrids were fewer and the central body less extensive (fig. 9).

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2. Black yolk chromatophores

For the first few days of their existence the black yolk chromatophores of both hybrids were identical with those of the pure F. heteroclitus in shape and the differences in size could apparently be entirely accounted for by differences in the ages of the cells. Compare figs. 13 and 14, 18 and 19 for the resemblance between the pure F. heteroclitus and the F. heterolcitus egg hybrid and figs. 8 and 11 for the resemblance between the pure F. heteroclitus and the F. majalis egg hybrid. In the pure F. majalis these cells were on the whole smaller and with more and longer processes than in the other three forms (see figs. 10, 12). This difference in F. majalis was most pronounced in those cells which were nearest to the embryo, as in fig. 10; the cells that were farther from the embryo (fig. 12) differed less in shape and size from those of F. heteroclitus.

We have seen that for the early stages there was complete dominance of the F. heteroclitus condition, as has been stated by Newman.[4] With further development, however, characteristic differences appeared so that each form could be distinguished by the appearance of the black yolk chromatophores alone.

In the pure F. heteroclitus these chromatophores soon crept onto the blood vessels, forming a coarse reticulum in which the clear spaces between the chromataphores were in general equal to or wider than the space occupied by the chromatophores themselves. There were very few branches traversing these clear spaces.

In the F. heteroclitus egg hybrid the chromatophores did not hug the vessels so closely so that the width of the colored meshes was usually about twice the thickness of the clear spaces between them. These clear spaces were also usually thickly traversed by a feltwork of fine processes (fig. 23). The color of this reticulum was also paler than in the pure F. heteroclitus, apparently because the chromatophores were spread over a greater area.

In the F. majalis egg hybrid there is, on account of the greater size of the egg, a greater space to be covered by the chromatophores. Together with this factor there was a much less pro-

[4] Jour. Exp. Zool., vol. 5, p. 550.

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nounced tropism of the chromatophores for the blood vessels, so that here no characteristic chromatophore reticulum was formed. In addition there was much variation in the shape of the chromatophores during these later stages; some of them still retained their previous characteristic polygonal shape, while others developed long processes like the red yolk chromatophores or the black yolk chromatophores in the pure F. majalis.

In pure F. majalis the black yolk chromatophores were characterized by their long and numerous processes also during these later stages.

In considering these later deviations from the complete dominance of the early stages it might be thought that we had here two intermediate conditions manifesting themselves in the hybrids due to a delayed influence of some 'branching factor' derived from F. majalis. For in both cases the deviations are in the direction of the more profuse branching characteristic of F. majalis. I think, however, that in this case the deviations from complete dominance are due mainly to a diminution of the tropism of the chromatophores for the blood vessels discovered by Loeb.[5] This reduced chemotropism is probably due to a change in the contents of the blood vessels resulting from the diminished yolk assimilation in the hybrids which will be discussed later. However, no matter what its cause, there can be no doubt that in both the hybrids the yolk chromatophores are less closely approximated to the blood vessels than in either of the pure forms. Now when in

[5] Jour. Morph. 1893, viii, 161; Arch. f. d. ges. Physiol. liv, 525. Loeb at first thought that this tropism was chemotropism due principally to oxygen. Later he was inclined to consider that it might be stereotropism. Among the embryos in this series it was noticed in a number of cases that after the circulation had become well established and the chromatophore network was well formed that the blood accumulated in some one region and stopped circulating through the vessels, although the heart still continued beating normally for several days. When this stoppage of the circulation took place in a comparatively early stage it was seen on several occasions that the chromatophores left the vessels and became again uniformly distributed over the surface of the yolk. As the vessels were still present it must be concluded that chemotropism and not stereotropism is responsible for the creeping of the chromatophores on to the blood vessels. When the circulation stopped in later embryonal life the chromatophores usually continued to remain on the blood vessels even several days after the blood had ceased to circulate.

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pure F. heteroclitus this chemotorpism of the chromatophores for the blood vessels is destroyed by the stoppage of the circulation the black chromatophores of this form also develop more branches than usual and may even in extreme cases (such as fig. 24) simulate closely the chromatophores of the pure F. majalis. This instance shows very clearly the importance of an analysis of the mechanism of heredity insisted upon by Loeb in 1898[6] and more recently by Newman.

3. *Head chromatophores*

One of the factors which influenced Newman in arriving at the conclusion that in the hybrids between majalis and heteroclitus, blended inheritance predominates is the manner of the appearance of the head pigmentation. Newman found, and I have confirmed his results, that the head pigment appears first on the pure F. heteroclitus; next on the F. heteroclitus egg hybrid; next on the F. majalis egg hybrid; and last on the pure F. majalis. Now, while this is true, still a closer analysis of this process has furnished what is probably the clearest case of Mendelian dominance encountered in this whole study.

In the pure F. heteroclitus, and in both hybrids the black head chromatophores appeared in two crops separated by an interval of about two days. In the pure F. majalis, on the other hand, this first crop was wanting and the second crop appeared at about the same time that it did in the pure F. heteroclitus.

The first head chromatophores appeared on the first or second day after the first appearance of the yolk chromatophores. They were first seen on the sides of the brain, having probably migrated in from the yolk, and wandered onto the dorsum of hind and mid-brain, only in rare cases reaching the fore-brain. Various stages in the development of these cells are shown in figs. 1 to 6. As soon as they reached the dorsum of the brain they began to expand and finally became very conspicuous objects (fig. 5). Figures 2 and 3 which show these chromatophores before and after an interval of three and a half hours give some idea of the rapidity with which this migration and expansion takes place.

[6] Marine Biol. Lab. Lectures for 1897 and 1898, pp. 227–229.

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In both hybrids these head chromatophores appear in essentially the same way that they do in the pure F. heteroclitus; there are, however, minor differences. In the hybrids they appear a little later, and they remain for a day or more on the sides of the brain without migrating onto its dorsum instead of only for several hours as in the case of the pure F. heteroclitus. Even after the chromatophores of the hybrids have reached the dorsum of the brain they do not look like any stage in the development of the same cells in the pure F. heteroclitus, for they are smaller and there are more of them. The comparative numbers of these cells for ten fish each of the pure F. heteroclitus and the F. heteroclitus egg hybrid are given in table 2. The differences while not great are, I think, significant. In the F. majalis egg hybrid the size and numbers of these cells are essentially similar.

TABLE 2

		NUMBERS OF HEAD CHROMATOPHORES										AVERAGE
Pure F. heteroclitus	on mid-brain..	2	0	0	1	5	1	2	0	0	4	1.5
	on hind-brain.	5	4	3	4	4	4	3	2	0	5	3.4
F. heteroclitus egg hybrid.....	on mid-brain..	6	6	5	1	3	0	6	5	10	4	4.6
	on hind-brain.	5	4	2	3	4	7	4	6	3	4	4.2

In the pure F. majalis, on the other hand, there was no sign of this first crop of head chromatophores except in one case, out of several hundred embryos examined; and in this case the first crop was represented only by a single cell. With this single exception the first chromatophores to appear on the head of F. majalis were similar in all respects to the second crop of the other forms.

The second crop of head chromatophores in the pure F. heteroclitus and the two hybrids always appeared at a distinct interval after the first crop. This interval varied from one to four days. In the hybrids this crop usually appeared one or more days later than in the pure F. heteroclitus; but in the pure F. majalis it appeared at the same time as in the other pure form. In all four forms the method of development of this crop was the same, and was different from that of the first crop. As far as could be seen

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these cells developed *in situ;* appearing first as faint, grey, thin and well branched cells scattered all over the dorsum of the brain. Soon after their first appearance their color became much deeper and they began to expand rapidly, so that after several days it was no longer possible to distinguish them from the chromatophores of the first crop. Fig. 6 shows the appearance of this second crop of head chromatophores in the same embryo which is represented nineteen hours earlier in fig. 5. A little later stage after the chromatophores of the second crop have become so large that they cannot always be certainly distinguished from those of the first crop is shown for the F. majalis egg hybrid of series 7 in fig. 9. To be compared with this last figure is fig. 10 taken from an embryo of the pure F. majalis also of series 7 and having the same age as the embryo figured in fig. 9. The entire absence of anything at all corresponding to the first crop of chromatophores is very evident.

It is very clear then that the F. heteroclitus character 'presence of the first crop of head chromatophores' dominates over the F. majalis character 'absence of this crop.' Furthermore, since the second crop of chromatophores appears in F. majalis synchronously with its appearance in F. heteroclitus, the delayed appearance of both crops in the hybrids cannot be considered a condition intermediate between that of the two pure forms.

4. Red chromatophores of the lateral line

In the pure F. heteroclitus and the F. heteroclitus egg hybrids at the time of hatching and the F. majalis egg hybrids (which usually do not hatch) at about the same time the lateral line is mapped out by a series of about twenty conspicuous red chromatophores the characteristics of which are shown in figure 27. In all of these three forms these cells were very similar in appearance, and there can be no doubt that they are essentially the same kind of cells as the red yolk chromatophores. In the pure F. majalis these red chromatophores were not present on the lateral line at hatching time. There was, however, a series of very

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indistinct pale cells showing white with a closed diaphragm which for a time I took to represent the red chromatophores. Later, however, it was found that these cells were probably the processes of the black chromatophores from which the pigment had withdrawn itself. For, all over fishes that were slightly older similar cells were found, with beautiful branched processes showing white by reflected light, and at the center of almost every one a small mass of contracted black pigment. We have then at the time of hatching what appears to be a clear case of the dominance of the F. heteroclitus character 'presence of red chromatophores' over the F. majalis character 'absence of red chromatophores.'

Immediately after hatching, however, this state of affairs began to change, for the red chromatophores began to fade, and when the fish were fed well had entirely disappeared in three or four days. When the fish were starved these chromatophores were visible in some cases for several days longer. These pigment cells did not contract or die but usually remained well branched and expanded as long as they were visible. The pigment, however, faded until it could only be seen in a few of the cell processes, usually lasting longest at the tips of these processes. Then it became practically invisible by transmitted, though still visible with reflected light; and finally could not be made out at all.

The possibility that in order to be visible this pigment needs something that it had been obtaining from the yolk but which is absent in the ordinary food naturally suggested itself, and perhaps receives some support from the fact that the pigment faded sooner when the fish was fed. But on the other hand, this rapid fading might equally well have been due to a general acceleration of development due to the feeding. An attempt was made to test the matter by feeding yolk but the close of the breeding season prevented conclusive results.

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5. *Black chromatophores of the lateral line*

The pure F. majalis upon hatching and shortly before hatching had a series of from 40 to 60 black chromatophores along the lateral line (fig. 25). There were usually two chromatophores to the segment. One of these was near the surface of the fish and expanded in a plane parallel to the surface. The other was situated farther from the surface upon the septum in the frontal plane separating the dorsal from the ventral musculature. This second chromatophore expanded in the plane of this septum.

The pure F. heteroclitus at hatching time and before, usually had no black chromatophores at all along the lateral line. Perhaps ten per cent had one or two black chromatophores along the lateral line, and a much smaller percentage had more. But none were seen which had more than ten or twelve black chromatophores in this place. Upon the first day after hatching, however, 80 per cent or 90 per cent of the fish were found to have black chromatophores varying in number from 1 to 26 and averaging about 8. During the next few days this increase continued until all the fish had from 20 to 30 black chromatophores along the lateral line.

The F. heteroclitus egg hybrids were on the whole similar to the pure F. heteroclitus, but exhibit a slightly intermediate condition as the black chromatophores begin to appear on the lateral line a little earlier than in the pure F. heteroclitus. Thus at hatching time the hybrids usually had enough chromatophores so that above the anus, when expanded, they made a continuous line of black, with scattered black cells posterior to this region. Fig. 26 represents part of a fish of this kind, in which, however, the chloretone given to quiet the animal has caused the chromatophores to contract slightly and thus break up the continuous black line which was originally present. In the same series a comparison of the lateral lines a few days after shows that at this time also the hybrids maintained their lead in the development of the black chromatophores along the lateral line. At that time ten of the hybrids averaged 29.2 black chromatophores to a

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lateral line with the extremes at 21 and 34; while ten of the pure form averaged 18.6 with extremes at 3 and 29. In this series the F. heteroclitus egg hybrids hatched at the same time as the pure F. heteroclitus so that these comparisons were made at the same age as well as the same stage and the difference in favor of the hybrids cannot be due to their greater age.

In the F. majalis egg hybrids the black chromatophores on the lateral line behaved quite similarly to those of the F. heteroclitus egg hybrid. But as these hybrids usually never do hatch a precise determination of the hatching time could not be made.

We have then so far as this character is concerned a well marked difference between the two species at the time of hatching and an incomplete dominance of the F. heteroclitus condition in the hybrids; but since the stages before and after hatching have not been sufficiently studied it cannot be told whether both species go through exactly the same series of changes, merely differing in their rate, or to what extent the hybrids are intermediate between the two pure forms.

6. *Distribution of yolk chromatophores*

At the time of their first appearance both kinds of yolk chromatophores in the pure F. heteroclitus and the F. heteroclitus egg hybrids were found to be distributed over the whole surface of the yolk, and the region opposite to the embryo had quite as many or nearly as many chromatophores as any other region. In the pure F. majalis, on the other hand, almost the whole of the yolk hemisphere opposite to the embryo was free from chromatophores; and it took a number of days before the migration of pigment cells into this region became noticeable. The F. majalis egg hybrids presented an intermediate condition, for most of them had a small chromatophore free area in the region opposite to the embryo, and the others had fewer chromatophores than usual in this region. Figs. 11 and 12 show something of the differences between these last two forms. Later on the migration of chromatophores filled these empty areas with pigment cells and obliterated the difference between the various forms.

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7. *Rate of development of yolk chromatophores*

In the pure F. heteroclitus the black yolk chromatophores first appeared when the heart was beginning to beat and before a circulation had been established; also before the fore-brain had acquired any lumen. The embryo had about twelve somites. The red chromatophores could usually not be seen until the next day.

In the F. heteroclitus egg hybrids the black yolk chromatophores also appeared at the time when the heart was first beating and before the circulation had started. In this form, however, the heart-beat and circulation started a little later than in the pure F. heteroclitus. At this time the fore-brain of the embyro usually had something of a lumen (condition was intermediate between figs. 1 and 2). Accordingly in this form the first appearance of the yolk chromatophores was later in time and also at a later stage in the development of the embryo.

In the F. majalis egg hybrids the yolk chromatophores did not appear until after the circulation was established, and until the embryo had a large lumen in the fore-brain like fig. 4.

In the pure F. majalis the yolk chromatophores appeared twelve to twenty-four hours later than in the F. majalis egg hybrids, at a time when the embryo was in a stage about half way between those represented in figs. 4 and 5.

Thus it is seen that both with respect to the time, and with respect to the development of the embryo, the hybrids had rates of development intermediate between those of their parent forms, and there was no indication of Mendelian dominance. The discovery, however, of factors which necessitated a Mendelian interpretation of the development of the head pigmentation, which, at first sight appeared exactly similar to this case of yolk pigmentation, makes one suspect that more study may result also in a Mendelian interpretation of the rate of development of the yolk chromatophores.

Although in this case the intermediate position of the hybrids and the lack of dominance is most evident, the same phenomenon is seen to a less degree in the development of the black chroma-

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tophores of the lateral line, and the arrangement of the black yolk chromatophores, where incomplete dominance is associated with differences in rate of development. Newman's results also point in the same direction, though he does not mention this contrast between the absence of dominance in characters connected with the rate of development and the presence of dominance in other characters. Thus most of the characters which Newman investigated were concerned with the rate of development and in most of them he found 'blended heredity.' Thus we see that in general characters connected with the rate of development show blended heredity, and it may be that such characters are so intimately connected with extra nuclear substances such as the yolk that complete dominance is not obtainable.

LATER DEVELOPMENT

This later development concerns only the two parent species and the F. heteroclitus egg hybrid for the F. majalis egg hybrid was never found to live longer than two days after hatching. Newman found that none of this form hatched; but in these experiments, usually a few fish hatched in each series, perhaps a dozen in all. In all of these the hatching seemed premature, the yolk sac had not been absorbed, and the fish died a little later.

In the other forms the chromatophores began to contract shortly after hatching, probably because they then came under the influence of the nervous system. As the amount of contraction varied with the environment, and with the condition of the fish, close comparison of color patterns, and of size and shape of the chromatophores was no longer possible. The motions of the fish, for it was usually not safe to risk narcotization, also made exact comparisions difficult, I think, however, that it may be safely said that the only changes that have taken place since hatching are all in the direction of making the three forms more like each other, until at the present time, three months after hatching I can find no characters which will distinguish any one form from the other two. They all have developed much more pigment of the black and greenish yellow kinds, especially in the dorsal region;

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and they all have developed from three to six or seven transverse black bands due both to an increased number of chromatophores and an increased expansion of the chromatophores in the region of the bands. Between the bands there are at present usually not more than one black chromatophore to the scale, while in the more pronounced bands there may be as many as four or five chromatophores to the scale. The present appearance of the fish is much like that of the females of F. heteroclitus shortly after the breeding season, when indistinct transverse dark bands may be seen.

CHARACTERS OTHER THAN THOSE OF PIGMENTATION

As regards the rate of the development of the embryo my observations confirm those of Newman on most points. The development of the F. heteroclitus egg hybrid was slower than that of its maternal parent; and the development of the F. majalis egg hybrid, during the early stages was faster than that of the pure F. majalis. After hatching the F. heteroclitus egg hybrid seemed more vigorous and grew faster under like conditions than either of the pure forms.

The failure of the F. majalis egg hybrids to develop well during the later stages seemed to depend primarily upon the poor digestion of the yolk in this form. The first considerable difference that could be seen between the pure F. majalis and the F. majalis egg hybrid was that in the hybrid the yolk was not digested away from under the embryo as rapidly as in the pure form. A result of this appeared to be that, when the heart was forming, the vesicle underneath the embryo was very shallow and the normal anterior curvature of the head did not take place (compare figs. 28 and 29). These two factors seemed to be responsible for the fact that when the heart first began to beat in the hybrid it was closely pressed to the ventral side of the embryo (fig. 28) and did not extend across the vesicle making an angle of nearly 90° with the embryo as in the pure form (fig. 29). Consequently from the very first the heart in the hybrid was much stretched and on the next day when the circulation had become established the heart

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in the hybrid was much longer, narrower, and less efficient than in the pure form. In the hybrid the heart never did get over this initial handicap but was stretched farther as development proceeded until finally shortly before the embryos died it had assumed the appearance shown in fig. 30, and at each beat was propelling only a very small amount of blood through the vessels. It seems then that a slight retardation in the digestion of the yolk led to such an increase in the distance between the points of attachment of the heart to yolk sac and embryo, that the heart could not grow fast enough to catch up, but remained permanently disabled.

SUMMARY

1. While it had been generally assumed that in hybrid embryos the inheritance was either maternal or paternal, Loeb, King and Moore have called attention to the fact that for the hybrid sea-urchin embryo we find dominance of individual characters as in the adult. For Fundulus hybrids Newman found a dominance of a few individual characters, but usually found the characters intermediate between those of the two parent species. In this study, which is concerned mainly with the pigment characters of Fundulus heteroclitus, F. majalis, and their hybrids, dominance of individual characters has been found in most cases, as in the following characters:

a. The character—presence of many large red yolk chromatophores (F. heteroclitus condition) is dominant over the character—presence of few small red yolk chromatophores (F. majalis condition).

b. The size and shape of the black yolk chromatophores of F. heteroclitus is dominant over the size and shape of these same cells characteristic of F. majalis.

c. The presence of a first crop of head chromatophores appearing before the majority of the head chromatophores (F. heteroclitus condition) is dominant over the absence of this crop of head chromatophores (F. majalis condition).

d. The presence of red chromatophores along the lateral line at hatching time, or shortly before it (F. heteroclitus condition)

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is dominant over the absence of red chromatophores at the same time (F. majalis condition).

2. In all of the above characters which concern mainly the presence or absence and not the time relations of the pigment characters the dominance is very evident, though often to a certain extent incomplete.

3. In the following characters, however, which are mainly concerned with the time relations the dominance is much less complete, or wanting altogether.

a. At hatching time F. majalis has a row of 50 or 60 black chromatophores along the lateral line, while in F. heteroclitus there are usually no chromatophores on the lateral line until hatching time when they begin to appear and gradually increase in number. The hybrids are intermediate, having at hatching time about 15 or 20 black chromatophores on the lateral line, and developing additional cells more rapidly than in the pure F. heteroclitus.

b. When the yolk chromatophores in F. heteroclitus first appear they are evenly distributed over the whole yolk sac; while in F. majalis they are absent from the yolk hemisphere farthest from the embryo. The F. heteroclitus egg hybrid is like its maternal species, while the F. majalis egg hybrid is intermediate having, on the side of the yolk sac opposite to the embryo, a small area in which the chromatophores are either absent or fewer than elsewhere.

c. A more perfect case of blended inheritance exists, as Newman has already shown, for the time of appearance of the yolk chromatophores. In F. heteroclitus these cells appear much earlier (both with respect to time, and with respect to the stage of development of the embryo) than in F. majalis. In the hybrids the time of appearance of these cells is intermediate, but each hybrid resembles its maternal more than its paternal parent.

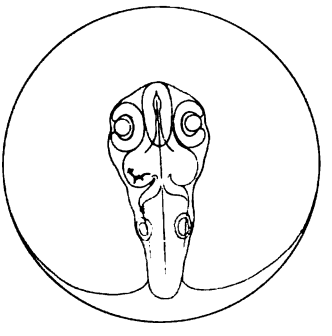
4. It appears then that the presence of certain pigment characters dominates over their absence or lesser development; while for the time relations of these pigment characters blended heredity holds. This difference may be fundamental or due to an incomplete analysis of the time relations.

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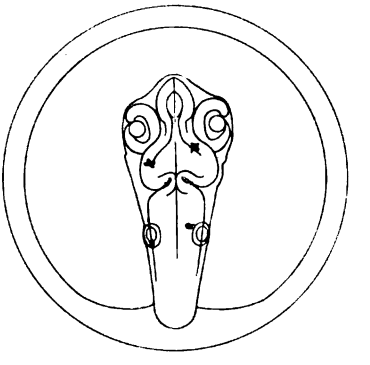
5. A similar case of blended inheritance, described by Newman for the time of first appearance of head pigmentation, was found to be actually a case of the combination of two crops of head chromatophores. The second crop appears in both species and hybrids The first crop is present in F. heteroclitus and both hybrids and hence its presence is a dominant in the Mendelian sense.

6. Immediately after hatching the characters which have served to distinguish the four forms begin to disappear so that after a few months both pure species and the hybrids look practically alike.

All camera drawings from living fish. The opaque red chromatophores are figured in red. Dotted lines indicate blood vessels. H = heart.



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Fig. 1 Pure F. heteroclitus, four days old, showing first chromatophores. × 25.

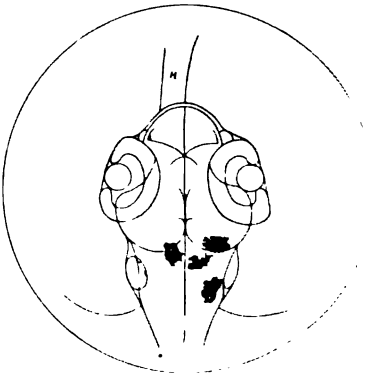
Fig. 2 Pure F. heteroclitus, three and one half days old, circulation started not more than a few minutes before drawing began. × 25.

Fig. 3 Brain of same embryo drawn in fig. 2 but three and one half hours later. × 25.

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Fig. 4 Pure F. heteroclitus, five days old, showing the brain chromatophores and half of the yolk chromatophore ring about the embryo. H = heart. × 25.

Fig. 5 Pure F. heteroclitus, six days old, showing the first crop of brain chromatophores well expanded. Red brain chromatophores not drawn. × 25.

Fig. 6 Same embryo drawn in fig. 5, but nineteen hours later showing the first appearance of the second crop of brain chromatophores. The four cells of the first crop still recognizable. × 25.

Fig. 7 Same embryo drawn in fig. 6, but forty-eight hours later. Shows the fusion of the head chromatophores.

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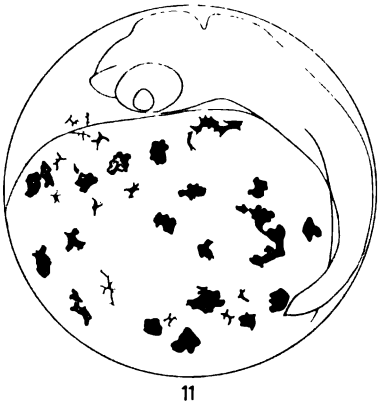
Fig. 8 Pure F. heteroclitus, three and one-half days old. Heart beating but no circulation. Shows the yolk chromatophore ring about the edge of the hollow vesicle under the embryo. × 25.

Fig. 9 F. majalis egg hybrid, seven days old. Shows both crops of head chromatophores. × 25.

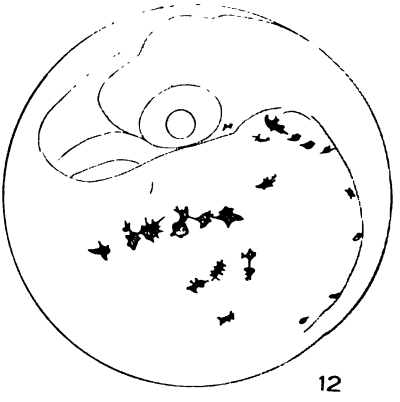
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Fig. 10 Pure F. majalis, seven days old. Belongs to same series as embryo of fig 9. Shows absence of the first crop of head chromatophores; also characteristic shape of black yolk chromatophores. × 25.

Fig. 11 F. majalis egg hybrid, six days old. Shows shape and arrangement of yolk chromatophores. × 19.

Fig. 12 Pure F. majalis, six days old. Shows the absence of yolk chromatophores on the part of the yolk away from the embryo. Many chromatophores near the embryo were not drawn as they were much obscured by the yolk globules. ×19.

Fig. 13 Pure F. heteroclitus, four days old. There were no blood vessels near the cells figured. Shows characteristic early chromatophore group, before they have migrated onto the blood vessels. Embryo same stage as fig. 2. × 114.

Fig. 14 F. heteroclitus egg hybrid, four days old; has good circulation. Embryo same stage as fig. 2. Shows characteristic group of yolk chromatophores on the side of the yolk sac, opposite to the embryo, where the blood vessels have just become established. × 114.

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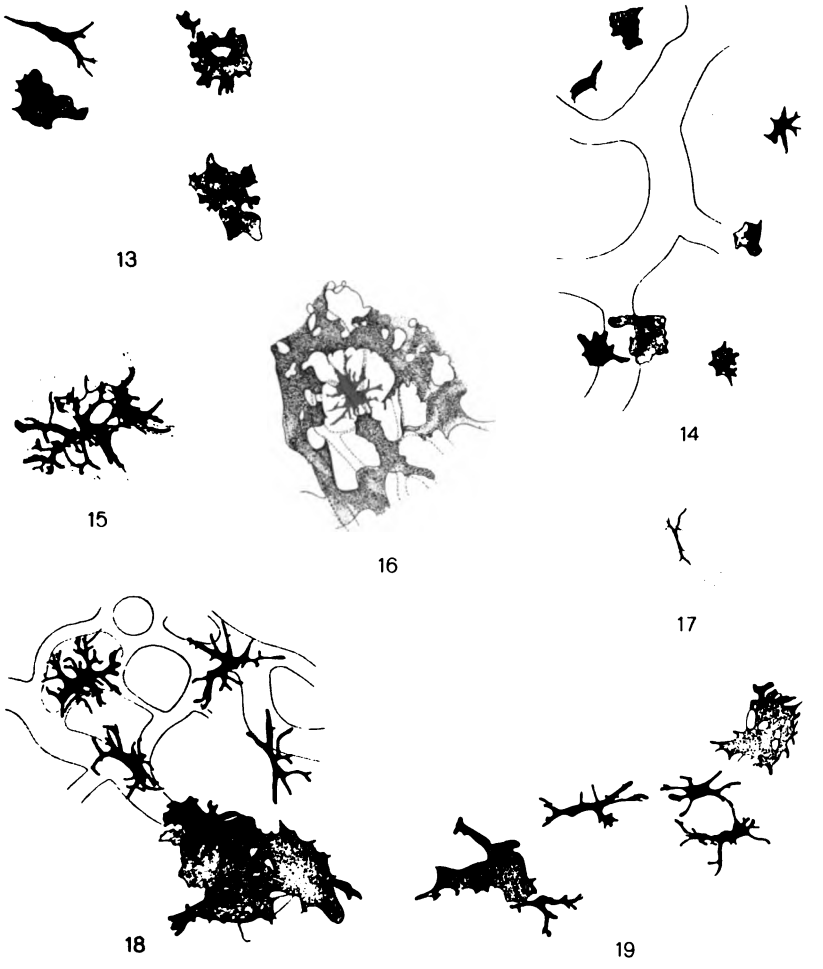


Fig. 15 Pure F. heteroclitus, twelve days old. Fully developed red chromatophore near the eye of embryo. Dotted lines indicate blood vessels. × 75.

Fig. 16 F. heteroclitus egg hybrid, twelve days old. Fully developed red yolk-chromatophore in center of clear space in black chromatophore reticulum. × 75.

Fig. 17 Pure F. majalis, thirteen days old. Characteristic red yolk chromatophore on blood vessel. × 75.

Fig. 18 Pure F. heteroclitus, six days old. Group of chromatophores beginning to form chromatophore reticulum on blood vessels near left eye. × 114.

Fig. 19 F. heteroclitus egg hybrid, six days old. Same series and same age as embryo of fig. 18. Group of chromatophores on blood vessel near left eye. × 114.

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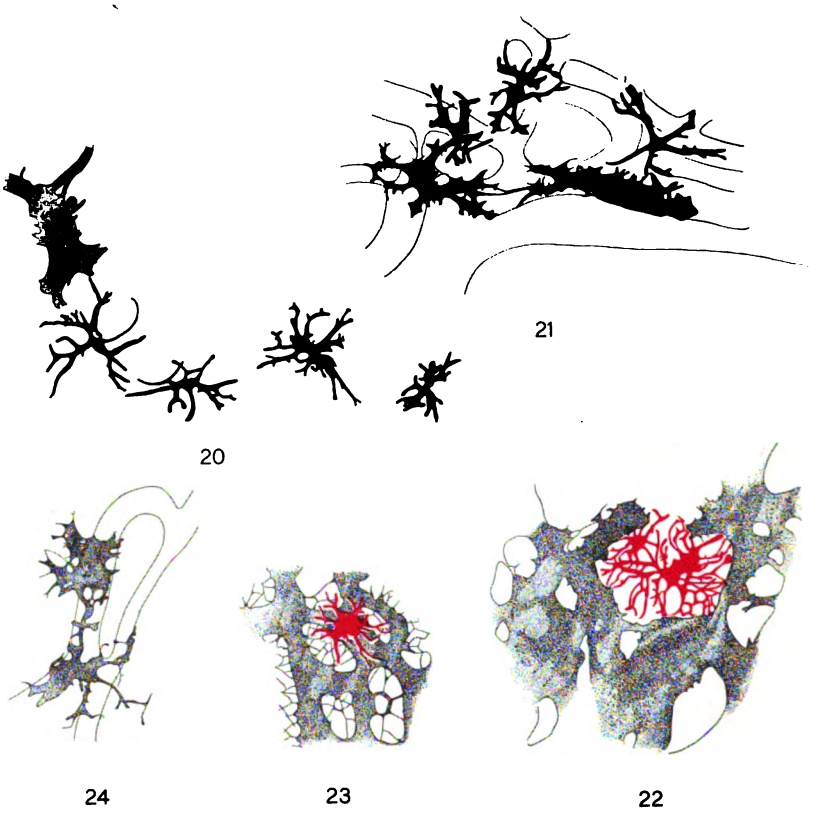


Fig. 20 Pure F. heteroclitus, seven days old. Same embryo as in fig. 18. Chromatophores on blood vessel near left eye. To show increase in size and complexity during last twenty-four hours. × 114.

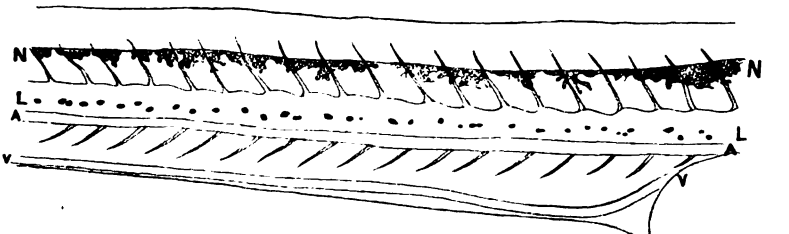
Fig. 21 F. heteroclitus egg hybrid. Same embryo as in fig. 19. Chromatophores on blood vessel near left eye. Note increase in size of red chromatophores and the beginning of the small processes of black chromatophores. × 114.

Fig. 22 Pure F. heteroclitus, eleven days old. Typical fully developed red chromatophore among the black chromatophore reticulum. × 114.

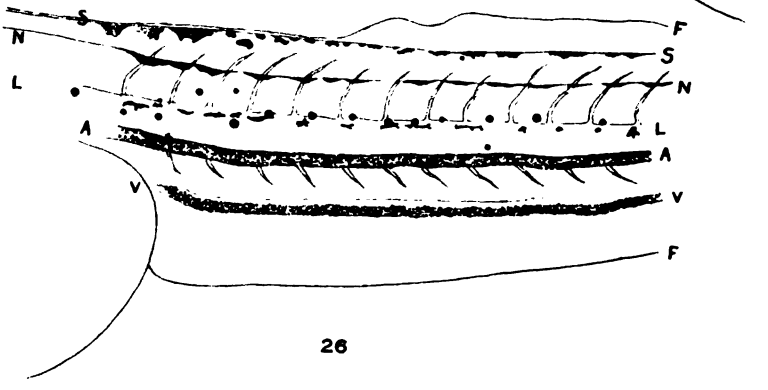
Fig. 23 F. heteroclitus egg hybrid, eleven days old. Of same age and series as embryo in fig. 22, with which it is to be compared. Note double layer of chromatophores and fine black reticular processes. × 114.

Fig. 24 Pure F. heteroclitus. Most of the eggs of this lot had hatched, but in this embryo the circulation had stopped though the heart was still beating, and the blood vessels distinct and full of blood. The yolk chromatophores have begun to lose their arrangement on the blood vessels and have developed much longer branches than any normally seen in this species. × 114.

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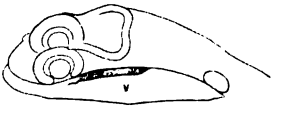
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Fig. 25 Pure F. majalis, thirty-five days old, just hatched. Shows the line of chromatophores along the dorsal surface of the nerve cord, *N*...*N*, which were expanded; and the contracted chromatophores of the lateral line, *L*...*L*. *A*...*A* = Aorta, *V*...*V* = Ventral vein. Neither the chromatophores on these last two vessels nor on the dorsal surface of the fish have been drawn in. × 25.

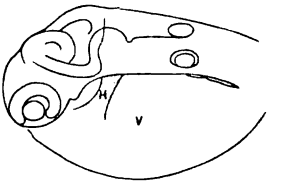
Fig. 26 F. heteroclitus egg hybrid, sixteen days old, just hatched. *S*...*S* line of chromatophores under dorsal skin, partly contracted by the narcotization. *N*...*N* chromatophores on nerve core, partly contracted; *L*...*L* lateral line. All the red chromatophores contracted, black chromatophores partly contracted at left where they formed a complete line when the drawing was begun. In the center of the lateral line the chromatophores are expanded; and on the right some of them are completely contracted on account of another dose of narcotic. *A*...*A* = Aorta *V*...*V* = Ventral vein. Chromatophores on these last two vessels drawn in from another uncontracted fish. × 25.

Fig. 27 Pure F. heteroclitus, just hatched. Shows red opaque chromatophores of the lateral line. × 127.

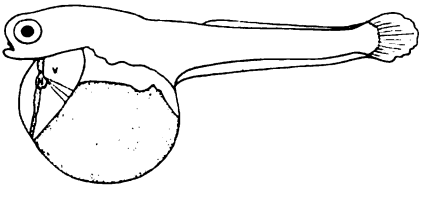
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Fig. 28 F. majalis egg hybrid, four days old. Shows position of the heart which has just begun to beat, and the shallow vesicle, V. underneath the embryo.

Fig. 29 Pure F. majalis, four days old. Same series and age as embryo in fig. 28. To show the greater depth of the vesicle, V. under the embryo and the normal position of head and heart. Heart has just begun to contract.

Fig. 30 F. majalis egg hybrid, twenty-four days old. Dissected out of the egg shell at a time when a few fish of the same lot had hatched and when many had died. Shows vesicle V. under the embryo and the greatly stretched heart within it.

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THE HISTOLOGICAL SIGNS OF RESISTANCE TO A TRANSMISSIBLE SARCOMA OF THE FOWL

By PEYTON ROUS, M.D., and JAMES B. MURPHY, M.D.

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THE HISTOLOGICAL SIGNS OF RESISTANCE TO A TRANSMISSIBLE SARCOMA OF THE FOWL.*

BY PEYTON ROUS, M.D., AND JAMES B. MURPHY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 36–38.

The nature of the processes that enable some individuals to prevent or overcome the growth of a tumor is as yet not understood. The associated histological phenomena are striking, but they admit of various interpretations. In brief, they are as follows: Retrogression of an established tumor,[1] in the absence of extraneous factors, such as hemorrhage and infection, occurs through a process of gradual degeneration and disappearance of its elements. Many lymphocytes collect at the edges of the mass, and also macrophages. A more or less dense connective tissue forms about it, and strands of the same tissue penetrate and eventually replace it. Tumor cells of like sort, grafted subsequently into the animal,[2] survive for some days and may proliferate slightly. But the graft, as a rule, fails to become vascularized, and soon degenerates.[3] Tumor tissue implanted in an animal naturally resistant,[4] whether by reason of species, variety, or individuality, survives for some days and may proliferate rapidly in a newly formed, vascular stroma; but very shortly it dies and is absorbed from the midst of a granulation tissue. In susceptible

* Received for publication, January 16, 1912.

[1] For the extensive literature, see C. Da Fano, *Ztschr. f. Immunitätsforsch.*, 1910, v, 1.

[2] H. R. Gaylord, G. H. A. Clowes, and F. W. Baeslack, *Med. News*, 1905, lxxxvi, 91; G. H. A. Clowes and F. W. Baeslack, *Med. News*, 1905, lxxxvii, 968; P. Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

[3] B. R. G. Russell, *Scientific Reports of the Imperial Cancer Research Fund*, 1908, No. 3, 341.

[4] P. Ehrlich, *loc. cit.*; B. R. G. Russell, *loc. cit.*; A. M. Burgess, *Jour. Med. Research*, 1909, xxi, 575.

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animals, on the other hand, it continues to grow; and unless the host possesses some degree of resistance, there is about the new tumor no marked cellular reaction, apart from the increase in blood-vessels and connective tissue incidental to its support. If the tumor grows slowly, a dense connective tissue containing many lymphocytes may form about it, especially at points of spontaneous healing.[5]

These are the phenomena in mammals. Most authorities hold that they are incidental to the actual processes of resistance; but Bashford and Russell allot to them a more immediate significance. According to these authors,[6] "Active resistance to an implanted tumor consists primarily in an inhibition of the specific chemiotactic powers which cancer cells exercise upon the connective and vascular tissues of the host," and the resistant individual exhibits resistance by a failure to supply to the tumor graft that vascularizing and supporting connective tissue framework which is supposed to be specifically elicited by the introduced cells, to be peculiar to each strain of tumor, and absolutely necessary to its development.

The present paper deals with the histological features of resistance to a transmissible spindle-celled sarcoma of the fowl.[7] Avian tumors have not heretofore been studied in this regard. The findings with respect to the sarcoma have a special interest owing to the presence in its tissue of a filterable agent which suffices to produce the tumor in new hosts. The appearance and behavior of the growth are those of a typical neoplasm; its cells themselves are transplantable, and it is best transferred to new hosts by means of grafts of the living tumor tissue.

METHOD.

We have employed chiefly a method much used for similar histological studies in mammals. A number of bits of the fresh tumor are inserted beneath the skin of each of several hosts, and with the tissue about them are removed on successive days. The histo-

[5] H. Ribbert, Das Karzinom des Menschen, Bonn, 1911, p. 195; C. Da Fano, *loc. cit.*

[6] E. F. Bashford and B. R. G. Russell, *Lancet*, 1910, clxxviii, 782.

[7] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397; *Jour. Am. Med. Assn.*, 1911, lvi, 198.

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logical changes can thus be traced from the beginning. The hosts used were: (i) susceptible; barred Plymouth Rock fowls in which, as the result of a previous inoculation, the sarcoma was already growing, or in which it developed from the engrafted tissue used for the observations; (ii) naturally resistant; (*a*) barred Plymouth Rock fowls in which the sarcoma had failed to develop on a previous implantation; (*b*) chickens of unfavorable variety; (*c*) ducks and pigeons; (iii) artificially resistant; barred Plymouth Rock fowls in which a tumor had retrogressed.

As a rule, eight or ten bits, one to two millimeters in diameter, of the same fresh tumor tissue were separately implanted in each of four or five fowls of the various kinds. The location of the grafts was marked on the skin with carbol-fuchsin. By means of a trocar, they were placed in the subcutaneous tissue of the feather-free strip between wing and thigh (lateral apterium), except when otherwise stated. Here they were visible and easily reached for removal. One or two were always left *in situ* to indicate the host's final state as regards resistance. Wound infection was not met with. The grafts were cut in serial section. A number of retrogressing tumors were also examined.

THE FINDINGS IN SUSCEPTIBLE HOSTS.

Grafts have been taken in series from six fowls carrying tumors, and from seven normal fowls in which susceptibility was proved by the fate of grafts left undisturbed. The fragments implanted in the six tumor-bearing hosts consisted of bits of their own neoplasm, as obtained by partial removal. Thus not only isotransplantation, but what may in a sense be called autotransplantation, was practiced.

The principal phenomena about the graft during the first forty-eight hours are those due to the trauma of its insertion. The tumor fragment, unchanged in appearance, lies in a rent in the host tissues, and exudation and a moderate accumulation of polymorphonuclear leukocytes occur about it (figure 1). The host tissues are edematous at the torn margin, the blood-vessels dilated, and toward the end of the period fibroblasts and macrophages appear. During the next twenty-four hours (third day), the tumor often unites at one or more points with the host tissues, and although unvascularized, is proliferating, and already may be much increased in size (figure 2). Its loose structure facilitates

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nourishment by a direct interchange of fluids; but, unless the bit is broken or very small, a necrosis takes place at the center. Cell division is mostly by amitosis. The polymorphonuclear leukocytes are now disappearing; but in some hosts small round cells, histologically identical with the lymphocyte, begin to collect in small numbers, localizing at points where the tumor has become attached to the host tissue. In this latter, fibroblasts, macrophages, and abundant newly formed capillaries are seen, with perhaps one or two foreign body giant cells enclosing debris from the trochar. By the fourth day, or later, the graft has markedly increased in size, and is vascularized (figure 3). But often before vascularization occurs, the tumor cells are invading the host tissue at points where they have united with it. Proliferating strands of the spindle-shaped elements can be seen extending out into the normal structures, utilizing for support the blood-vessels and connective tissue already present. At the advancing margin, lymphocytes become fewer, and in very susceptible hosts they may be absent, as is indeed all cellular reaction. The growth may now be considered as established, and for present purposes need be followed no further.

In general, these processes appear to take place more rapidly in the fowl furnishing the engrafted tissue. Certainly in these hosts which already carry a tumor very few lymphocytes collect about the graft. The proliferative activity of the neoplastic cells, once the tumor has become established, is sometimes startling. As many as 46 per cent. of them may be in process of division at one time.[8] The extension and enlargement of the sarcoma from this source is very obvious. The findings do not in the least suggest that its growth can take place in any other way.

The process of transplantation becomes almost diagrammatically evident when the grafts have been placed in the pars papillaris of the corium (figures 1, 2, and 3). The epidermis of the fowl is so loose, transparent, and filmy, and so easily stripped away, that macroscopically it might well be mistaken for a keratinized layer elaborated by deeper epithelial cells. Grafts placed just under it by means of a blunt trocar are sharply defined, and their least increase in size is at once seen. Microscopically, the sarcoma cells contrast sharply with those of the surrounding structures. The leukocytic and proliferative response are here at a minimum; and the tissues do not lend themselves to invasive growth. For these reasons we have in general preferred subcutaneous sites, and have made intradermal implantations only in special demonstration of the transplantability

[8] James B. Murphy and Peyton Rous, *Jour. Exper. Med.*, 1912, xv, 119.

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of the tumor cells and the development of the new sarcoma from them.

The features mentioned may be further illustrated by an example accidentally come upon. Several fowls, dying of intraperitoneal growths caused by the injection into the abdominal cavity of a suspension of the sarcoma cells, showed curious tumor formations on the surface of the liver. They were discrete, glistening, of dew-drop shape and nearly as fluid, translucent, grayish, homogeneous, and without evident blood-vessels. They differed markedly from the larger, compact nodules which everywhere studded the peritoneal surface of the viscera. On section, they proved to be implantation growths, consisting of a very loose, non-vascularized, branching cell reticulum, attached superficially to the liver surface, but without an intimate connection with it (figure 4). They closely resembled growths *in vitro* in plasma, and presumably had received their sole nourishment from the mucinous fluid filling the peritoneal cavity.

Lastly, while discussing the origin of the sarcoma from transplanted cells, we may briefly cite here the fate of fragments of the growth injected directly into the venous blood stream. In favorable fowls, the injection is followed by the appearance of sarcomatous nodules in the lungs; and serial sections show very clearly the origin of these by a direct outgrowth from emboli of tumor cells.

We have not followed systematically the fate of the non-malignant stroma and the blood-vessels, introduced with grafts of the sarcoma. The morphology of the growth is such as to render very difficult observations of this sort; and they have not seemed essential, in view of the fact that before the graft itself has become vascularized, its cells may have already begun a rapid invasion of the host tissues, utilizing for support the preëxisting structures. Certain very invasive human sarcomata make a similar use of normal structures.[9]

THE FINDINGS IN NATURALLY RESISTANT FOWLS.

The sarcoma is most readily transmitted to chickens of the barred Plymouth Rock variety, the breed in which the original neoplasm

[9] J. G. Adami, Principles of Pathology, Philadelphia, 1908, p. 700.

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was found; but not uncommonly individuals fail to develop a tumor even on repeated implantation. Natural resistance is more frequent in chickens of other varieties, which, indeed, until the growth had reached its present high malignancy, proved entirely insusceptible. Grafts in series have been taken from four Plymouth Rock fowls with a natural resistance that was absolute, and from two Rhode Island Reds. Numerous less systematic observations have been made on resistant fowls of mixed breed. The findings were similar in all.

During the first forty-eight hours, the phenomena about the grafts do not differ from those in susceptible fowls. The fragment lies free in a rent in the host tissue, polymorphonuclear leukocytes collect about it, and exudation occurs. Following this period, death of the graft takes place in one of two ways: (*a*) Often it remains for many days unvascularized and almost unattached to the host tissue. During this period it undergoes a gradual necrosis. The marginal tumor cells are the last to succumb. They sometimes live for a week or ten days, and at first may multiply slightly, but they do not invade at the points of contact with the host tissue. Meanwhile the leukocytes disappear, fibroblasts and macrophages appear, and lymphocytes collect in sparse numbers, infiltrating the zone of living tumor. By the end of two weeks, as a rule, the engrafted tissue is wholly necrotic and its reorganization has begun. (*b*) More frequently, the tumor fragment grows, invades, and becomes vascularized with the same rapidity as in susceptible hosts. But immediately upon its attaining a foothold in the host tissues, lymphocytes appear and rapidly become very numerous, at first about the neighboring blood-vessels, then at the edge of the sarcoma, infiltrating to some degree its substance. As early as the fifth day, the graft may be embedded in a dense mass of small, round cells, and is already degenerating, despite its blood-vessels (figure 5). The spindle cells become hydropic, their nuclei stain poorly, and in the course of a few more days all are dead. The lymphocytic mass may be six or eight times the diameter of the foreign tissue it encloses, and is often so largely composed of small round cells as to suggest true lymphoid tissue. Fibroblasts and macrophages occur, but no giant-cells, except about foreign bodies other than the tumor tissue. The mass is supplied by numerous small vessels. Sometimes blood sinuses exist at its edge.

The two very different sets of phenomena leading to death of the graft may be observed in one host, a fact suggesting that local conditions largely determine their incidence. A tabulation of the fate of each graft, as compared with the conditions at the point where it was implanted, has shown that such as are placed in regions rich in subcutaneous tissue, become established and perish later in the midst of a lymphocytic nodule, whereas those inserted in the loose, poorly vascularized web, separating skin and muscle, fail

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to establish themselves and gradually die. In a similar way at unfavorable sites, in hosts actually susceptible, the sarcoma sometimes fails to establish itself. The fate of grafts which have obtained a foothold would seem, therefore, a truer expression of the host's resistance.

The above findings essentially resemble those occurring in the mouse under similar conditions. When mouse carcinoma is implanted in naturally resistant individuals, it frequently establishes itself and grows for a brief period, but soon breaks down in the midst of a granulation tissue containing lymphocytes.[10] The significance of the vastly greater accumulation of these cells in the chicken will be discussed further on.

THE EFFECTS OF PARTIAL RESISTANCE.

Natural resistance to the chicken sarcoma is often insufficient to protect the host completely. We have examined series of grafts from three Plymouth Rock fowls in which tumors slowly arose from the material left *in situ*, and we have made many less systematic observations upon large tumors that slowly followed implantation in chickens of other varieties. Doubtless in some of these, the character of the implanted material was responsible for the slow growth, while in others an induced resistance was present. The phenomena observed were identical with those already described, except that after the tumor tissue had established itself, it did not die, but slowly continued to proliferate. The lymphocytic accumulation at the growing edge remained well marked. About the long established growths, lymphocytes were plentiful, in association with a more or less well defined connective tissue overgrowth and encapsulation.

RESISTANCE IN FOWLS OF OTHER SPECIES.

The tumor will not grow in mammals or in other birds than chickens.[11] Its fate has been studied in grafts removed in series from three ducks and four pigeons. The findings in the two species were the same.

[10] B. R. G. Russell; A. M. Burgess; C. Da Fano.
[11] Peyton Rous, *loc. cit.*

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The implantation regularly gave rise, within a week or ten days, to a nodule, which was sometimes larger than that produced by the material in susceptible hosts. It was firm, sharply defined, elastic, and often one centimeter broad within a week after the implantation of a tumor bit one tenth this size. But soon its enlargement ceased, and within three or four weeks it had quite disappeared. Exceptionally a small, indurated lump remained longer.

Sections show that in practically all cases the tumor bit rapidly established itself and began to proliferate. Lymphocytes collected round about, and by the sixth day were present in immense numbers, forming the greater part of the nodule, and infiltrating the already hydropic, degenerating tumor tissue. Within a day or two death of the tumor cells almost *en masse* had taken place. The nodule persisted for some time longer, but there remained in it only a few necrotic strands to represent the graft. For the rest, it consisted of lymphocytes, with a few fibroblasts, macrophages, and polymorphonuclear leukocytes, and an occasional foreign body giant-cell, enclosing debris. Fibers of voluntary muscle included in the nodule atrophied even to complete disappearance. The mass was gradually absorbed.

The findings differ from those in naturally resistant chickens only in the regularity with which the graft established itself, in the greater lymphocytic reaction, and in the more abrupt death of all the tumor. They resemble those seen in mammals implanted with mammalian tumor from another species. For example, mouse tumor in the rat establishes itself, grows for a short period, and then abruptly dies in the midst of a granulation tissue.[12]

THE FINDINGS ABOUT TUMORS THAT RETROGRESS.

Retrogression of the well developed sarcoma is infrequent in healthy chickens. The first sign of it is a hardening of the tumor mass, very evident on palpation; then comes a cessation of growth; and at length a diminution in size. The mass disappears slowly and sometimes persists for months as a minute, sharply circumscribed nodule.

The initial hardening is due to a connective tissue overgrowth which becomes more and more marked as time goes on. A poorly vascularized, fibrous capsule forms about the neoplastic mass which is further divided into irregular lobules by fibrous septæ. A muci-

[12] P. Ehrlich, *loc. cit.;* B. R. G. Russell, *loc. cit.*

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nous degeneration of the tumor tissue gradually ensues; its cut surface becomes translucent, and a ropy fluid containing true mucin may be scraped away from it. Sometimes the center of the mass becomes cystic. Histologically, one finds lymphocytes at its edge, both in nodes and scattered irregularly in the capsule, septæ, and, to a less extent, in the marginal tumor tissue. This latter degenerates little by little and is absorbed, until at last there remains only a small, encapsulated group of spindle cells, with much mucinous, intercellular material (figure 6). Often the tumor cells are multinucleated or show a nuclear hypertrophy, budding, or chromidiosis. Similar appearances in human tumors are interpreted by Howard and Schultz[13] as indicating an attempt at regulation in depressed cells. Finally all trace of the tumor disappears.

The phenomena about the mass are similar to those associated with the retrogression of tumors in man and other mammals.[14]

THE FINDINGS IN CHICKENS WITH AN ACQUIRED RESISTANCE.

The retrogression of the chicken sarcoma induces a resistance in the host; for now grafts fail to give rise to a tumor, whereas formerly one was produced.[15] An acquired resistance in rats and mice, following out of like conditions, has long been recognized.[16] In the chicken, as in these mammals,[17] the resistance to the tumor strain that retrogressed is usually well pronounced, for some time at least, and often is not overcome by the introduction of large quantities of active tumor tissue. We have examined grafts in series from four fowls tested repeatedly in this way after retrogression of a sarcoma, and from three others in which the tumor, at one time four to six centimeters in diameter, had recently disappeared. In one of these latter, the resistance was not complete, as shown by the fact that, from among several grafts left undis-

[13] W. T. Howard and O. T. Schultz, *Monographs of The Rockefeller Institute for Medical Research*, 1911, No. 2.

[14] C. Da Fano, *loc. cit.*

[15] Peyton Rous, *loc. cit.*

[16] H. R. Gaylord, G. H. A. Clowes, and F. W. Baeslack; C. Jensen; P. Ehrlich; E. F. Bashford.

[17] J. W. Jobling, *Monographs of The Rockefeller Institute for Medical Research*, 1910, No. 1.

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turbed, in this host, a sarcomatous nodule eventually developed. The other fowls were absolutely resistant.

In hosts with an acquired resistance, the graft very frequently fails to establish itself. The march of events is similar to that seen, less often, in naturally resistant hosts and sometimes even in susceptible ones. The bit of implanted tumor remains unvascularized, shows no tendency to invade the host tissue at the points where it has become attached, and in the course of a week or ten days undergoes a gradual necrosis that affects last of all the tumor cells most exposed by position to the action of the fluids of the host. The same set of phenomena has been described by Russell as occurring regularly in mice with an acquired resistance to implanted tumor,[18] and it is associated with the death of grafts of embryonic tissue in resistant hosts of the same species.[19]

But acquired resistance to the avian sarcoma does not manifest itself according to this fashion alone. At least half the grafts become established, proliferate, invade, and, a week or ten days after the implantation, die more or less abruptly. In the meantime immense numbers of lymphocytes have collected round about, just as in resistant hosts of other sorts (figure 7). The death of the implanted tissue certainly cannot be laid to the failure to acquire a supporting and vascularizing stroma, as is assumed by Bashford and Russell[20] in explanation of the death of tumor tissue in hosts with acquired resistance.

THE LYMPHOCYTE AS AN EXPRESSION OF RESISTANCE OF THE HOST.

In resistant fowls of all kinds, large numbers of lymphocytes collect about the established tumor graft. About the retrogressing sarcoma also they are frequent. Several authors have noted the association of lymphocytes with mammalian tumors, and Da Fano[21] has concluded that they have a definite relation to resistance on the part of the host. He found them constantly about retrogressing

[18] B. R. G. Russell, *loc. cit.*
[19] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 344.
[20] E. F. Bashford and B. R. G. Russell, *loc. cit.*
[21] C. Da Fano, *loc. cit.*

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tumors, and about tumor grafts which had become established in resistant animals. When, on the other hand, the graft failed to become vascularized in the mammalian host, these cells were relatively few. They occurred even in susceptible individuals here and there about rapidly growing tumors. Our observations at all these points correspond with Da Fano's. But in mammals, the lymphocytic reaction is relatively inconsiderable; and it may well be asked whether the great accumulation of lymphocytes seen in the fowl is referable to the same cause, namely, to the tumor cells as such. The tissue of the avian sarcoma contains an extrinsic agent capable of giving rise to the growth; and the possibility that the lymphocytes are a response to this agent must be taken into consideration. Fortunately there are available several series of grafts of adipose tissue of the fowl which were removed by Dr. Tytler some days after their implantation in normal chickens. About some of these, lymphocytes accumulated in numbers comparable to those about the sarcoma in resistant hosts. Furthermore, Loeb and Addison[22] have described a lymphocytic reaction about pigeon skin implanted in the pigeon or chicken, and have remarked on its profuseness as compared with that seen about the same tissue implanted in a mammal (the guinea pig). From these facts, it seems justifiable to refer the reaction to the presence of the tumor tissue. In explanation of its profuseness, Loeb and Addison mention the large proportion of lymphocytes in the blood of the fowl. According to our experience they sometimes constitute in normal individuals over 40 per cent. of the white cells. It is known that they respond actively in the fowl to processes which in mammals affect them to a much smaller degree,—tuberculosis,[23] for example. About grafts of the sarcoma, all stages of an apparent emigration from the blood-stream can be seen; but to judge from a series of blood counts that we have made, no general lymphocytosis accompanies the striking local reaction.

In further elucidation of the lymphocytic response in resistant hosts, the findings about grafts of killed tissue have been studied.

[22] L. Loeb and W. H. F. Addison, *Arch. f. Entwicklungsmechn. d. Organ.*, 1911, xxxiii, 44.

[23] H. Hirschfeld and M. Jacoby, *Ztschr. f. klin. Med.*, 1909–10, lxix, 107.

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As the destructive agent, heat (55° C. for fifteen minutes), or repeated rapid freezing and thawing, has been used. Twenty series of grafts of killed or damaged tissue have been implanted and removed from susceptible or naturally resistant chickens.

About the bit of killed tumor no marked macroscopic reaction is seen. In a few days, it has diminished to a tiny yellow shred, often difficult to find. Later, with reorganization, the shred is converted into a translucent lump about the size of the original graft. Soon all is absorbed. Microscopically, there occur, first, a reaction to trauma, and then a reorganization of the necrotic tissue. Very few lymphocytes are present at any time. At most, a few small foci may be seen in the adventitia of the neighboring vessels. Experiments on the resistance of the extrinsic agent, elsewhere to be given in full, have shown that in fresh tumor tissue it will sometimes withstand a temperature of 50° or 53° C. for fifteen minutes, a degree of heat fatal to the tissue itself, as is shown by the latter's failure to grow in the plasmatic medium of Burrows,[24] as well as by following histologically the fate of the heated graft. The tumors which result from inoculation of the tissue are engendered by the extrinsic agent as such. The histological findings will be briefly described, since they illustrate the conditions that call forth a lymphocytic accumulation.

The tumor tissue heated to 50° or 53° C. for fifteen minutes undergoes necrosis after its implantation and is soon converted into a soft, yellow shred. Its reorganization begins, and a week or ten days after the implantation, the first tumor cells make their appearance. Thin strands of dividing spindle cells become noticeable, extending this way or that, among the fibroblasts of the reorganizing mass. They are not arranged with relation to a central focus. Proliferating rapidly, they soon form a considerable mass and begin to invade the host structure. And simultaneously with the formation of the tumor, lymphocytes, practically absent heretofore, collect in greater or less quantity, depending on the resistance of the host. They localize about the sarcomatous tissue.

It is plain, therefore, that the lymphocytic reaction owes its pres-

[24] M. T. Burrows, *Jour. Am. Med. Assn.*, 1910, lv, 2057.

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ence to the tumor cells, not to the extrinsic agent; and that it is called forth only by the living tumor tissue. Da Fano states that a lymphocytic reaction is not seen about grafts of killed mouse tumor. In the chicken, the reaction occurs under the same conditions as in mammals and is peculiar only in its profuseness.

The connective tissue overgrowth associated with the retrogressing chicken sarcoma can be disposed of briefly. Histologically, it is quite similar to that accompanying the absorption of mammalian growths. Macrophages are present in both instances. But the plasma cell, upon which Da Fano lays much stress in its relation to tumor resistance, is not seen in the chicken.

RELATIVE ACTIVITY OF THE EXTRINSIC AGENT.

The presence in the sarcomatous tissue of an agent capable of causing a neoplastic change in the host cells is important as affecting the interpretation of the facts reported in the present article. Although the cells of the sarcoma can be transplanted, it is debatable whether the new tumor does not enlarge in part by a neoplastic change in its surroundings, and whether it does not owe most of its bulk to this source. On the latter point, a negative answer can at once be given. No feature of the tumor is so pronounced as its growth by the proliferation of cells already neoplastic. A statement has already been made of the great percentage of cells which may be in process of division at one time. Histologically, the enlargement of the sarcoma appears to take place entirely by this intrinsic proliferation; and the invasion and replacement of new structures are plainly referable to such a mode of growth. Only by special methods (heat, cold, drying, filtration) can an action of the filterable agent be demonstrated. It has seemed not impossible that the resistance of certain hosts might constitute a differential whereby this action could be brought out,—the transplanted cells dying in such animals, while the extrinsic agent survives and engenders the growth. Our observations do not support this idea. It may be stated as a general rule that when the host is so unfavorable to the implanted cells that they die, no tumor arises.

In susceptible hosts, the development of the sarcoma after an

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implantation appears strictly dependent on the condition that the implanted cells obtain a foothold.[25] Sometimes, in susceptible hosts, as already stated, the tumor tissue fails to live; and sections, as well as the macroscopic finding, prove the absence of a sarcomatous change about these dead grafts. So, too, not infrequently it happens that the implanted tumor fragment unites with the host tissue only at one end, while for the rest it remains for some days free in the rent caused by its implantation. In such cases, the new tumor can be seen to develop at the point where the graft has become attached to the host tissue, and there only. In this connection, it should be stated that the extrinsic agent requires relatively a long time to engender the minutest palpable sarcoma,—at least one week, and sometimes more than a month.

From all these facts, it follows that the action of the extrinsic agent may be regarded as negligible in accounting for the phenomena seen within and about grafts of living tumor tissue shortly after their implantation.

REGARDING THE NATURE OF THE HOST'S RESISTANCE.

The significance which Bashford and Russell attach to the phenomena about grafts of tumor tissue has been briefly outlined. They hold that every transplantable tumor elicits from the susceptible host a specific, supporting, and vascularizing connective tissue stroma on which its growth depends. The findings with many slightly invasive mouse and rat tumors may be taken to support this thesis; since in hosts favorable to such growths, a "stroma reaction" for the support of the graft does undeniably occur, whereas in individuals with an acquired resistance, it is not evident under ordinary circumstances of observation, and the implanted tissue eventually dies. With grafts of hashed mouse embryo, as already mentioned, the same phenomena are witnessed. In these cases, the appearance or non-appearance of a stroma supporting the engrafted tissue does, to a striking degree, go hand in hand with its success or failure. But the findings with the chicken sarcoma

[25] In reality, this dependence is not absolute, as is shown by the results with grafts in which the tissue has been killed but the extrinsic agent survives.

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cannot be reconciled with the assumption that a specific stroma is absolutely necessary to the survival of the tumor, and that the resistance of the host finds its sole expression in the failure to supply this stroma.

Grafts of the chicken sarcoma in susceptible hosts do not always remain discrete and passive, so to speak, until a fresh organization with vessels and connective tissue enables them to grow. Well before the graft, as such, has been vascularized, very shortly indeed after its cells come in contact with the host tissue, an invasion of the host structures may begin. The neoplastic cells proliferate, and penetrate the surrounding parts, making use of the preëxistent vessels and connective tissue for their support, just as do the very invasive human sarcomata described by Adami. Furthermore, although it is true that in the chicken, as in the mouse and rat, tumor grafts, in hosts with an acquired resistance, often fail to be vascularized and eventually perish, yet death of the avian tumor does not always take place in this way. The implanted fragment may be abundantly vascularized, and may grow actively for some days before succumbing. The explanation might be advanced that the stroma reaction has suddenly failed the growth, which therefore can extend no farther; but this does not account for the death of such portions of it as are already excellently vascularized and supported; nor does it explain the retrogression of mammalian tumors.

These observations have formed the basis of a note by one of us,[26] in which was pointed out the insufficiency of the stroma reaction theory as a general explanation of the acquired resistance to implanted tumor. At the time of publication, the extrinsic agent capable of giving rise to the chicken sarcoma had not been discovered. Yet its recognition does not invalidate the conclusion then expressed; for, as we have shown, the phenomena occurring about grafts of the sarcoma are referable to the implanted neoplastic cells, and are similar to those observed with mammalian tumors. In a more recent paper, Goldmann[27] has independently come to the conclusion that the presence or absence of a stroma reaction is inadequate to explain the processes about tumor grafts

[26] Peyton Rous, *Jour. Am. Med. Assn.*, 1910, lv, 1554.
[27] E. E. Goldmann, *Beitr. z. klin. Chir.*, 1911, lxxii, 1.

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in rats and mice. Levin, too, has lately presented facts that point in this direction.[28]

The true nature of tumor resistance cannot here be profitably taken up. We are disinclined to attribute to the lymphocytes and connective tissue overgrowth, seen in mammals and birds alike, any fundamental importance in the process by which the host protects itself. These histological features are not notably different from such as accompany, as secondary phenomena, tissue derangements of many sorts.

CONCLUSIONS.

1. The living tissue of the chicken sarcoma, when implanted in susceptible hosts, survives, proliferates, and thus gives rise apparently to the whole of the new tumor. The histological findings about the graft do not suggest any other origin for the growth.

2. In fowls with a natural or acquired resistance, a very striking series of phenomena takes place about the graft or the established growth. They are referable to the presence of the living neoplastic cells and are essentially similar to those already described in mammals.

3. The lymphocyte has an association with the processes of resistance in the fowl, similar to, but more marked than that observed in mammals.

4. Resistance of the host does not constitute for the chicken tumor a differential means whereby the extrinsic agent present in the growth can be observed to engender it. In general, it may be stated that, when the new host is so unfavorable to the transplanted sarcoma cells as to cause their death, no tumor develops.

5. The activity of the extrinsic agent is negligible as affecting the interpretation of the phenomena about grafts of the avian sarcoma in resistant and susceptible hosts. They are referable to the presence of the transplanted cells.

6. The appearance or non-appearance of a specific, supporting, and vascularizing reaction in the host tissues cannot be looked upon as determining the fate of grafts of the chicken sarcoma. The same seems to be true of mammalian neoplasms.

[28] I. Levin, *Jour. Exper. Med.*, 1911, xiii, 604; 1911, xiv, 139.

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EXPLANATION OF PLATES.[29]

PLATE 36.

FIGS. 1, 2, and 3. The fate of the sarcomatous tissue in a susceptible host. The three grafts shown in cross section with the surrounding tissue were removed on the second, third, and fourth days, respectively, after their implantation between epidermis and corium (pars reticularis) in the same host. This host carried a tumor which was operated upon to obtain material for the implantation. The grafts were originally of the same size, and all have the same magnification.

In figure 1, the tumor bits lie free in a rent in the host tissue. In figure 2, they have grown markedly and are attaching to the host tissue, which, histologically, is sharply distinct from them. Vascularization has not yet occurred. In figure 3, the bits have united to form a nodule, the growth of which has already pushed to one side the adjoining structures.

PLATE 37.

FIG. 4. A sarcomatous growth attached to the surface of the liver. The fowl had received an intraperitoneal injection of a suspension of tumor fragments in salt solution. The mass is without vascularization. It markedly resembles a culture of the tumor cells *in vitro*, and apparently had received its sole nourishment from the peritoneal exudate.

FIG. 5. A portion of a tumor graft that established itself in the naturally resistant host (Plymouth Rock fowl) and began to grow. There is a dense accumulation of lymphocytes about the tumor tissue, which shows signs of degeneration. Fifth day after the implantation.

PLATE 38.

FIG. 6. Margin of a small nodule that remained for many weeks as the sole remnant of a large sarcoma that retrogressed (Plymouth Rock host). The encapsulation, the lymphocytic nodes and infiltration, and the degenerate forms of the tumor cells are characteristic.

FIG. 7. A portion of a tumor graft that had established itself and begun to grow in a chicken with acquired resistance. Fifth day after the implantation. There are many distended blood-vessels in the tumor tissue. They appear in the photograph as dark spots, owing to the deeply stained nuclei of the erythrocytes. About the tumor is a dense accumulation of lymphocytes. After a brief preliminary growth, the grafts left *in situ* all died.

[29] All the sections were stained with methylene blue and eosin.

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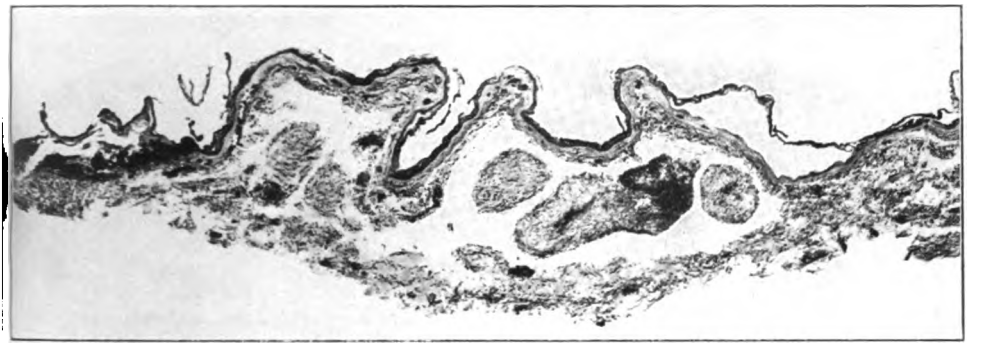


FIG. 1.

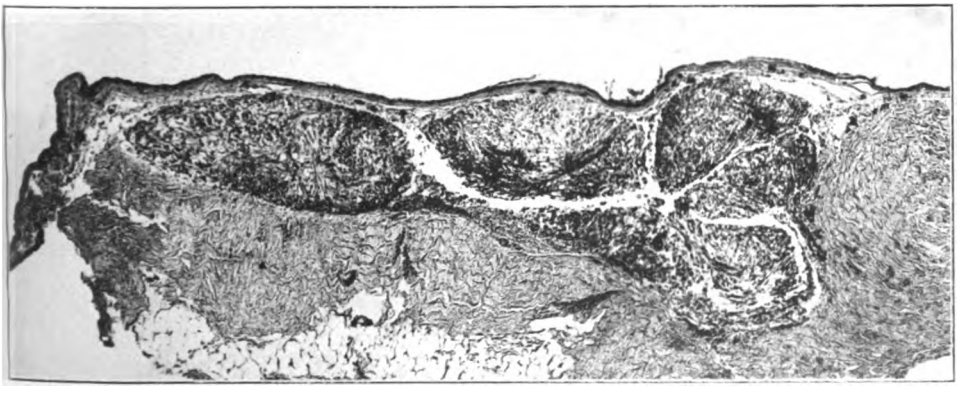


FIG. 2.

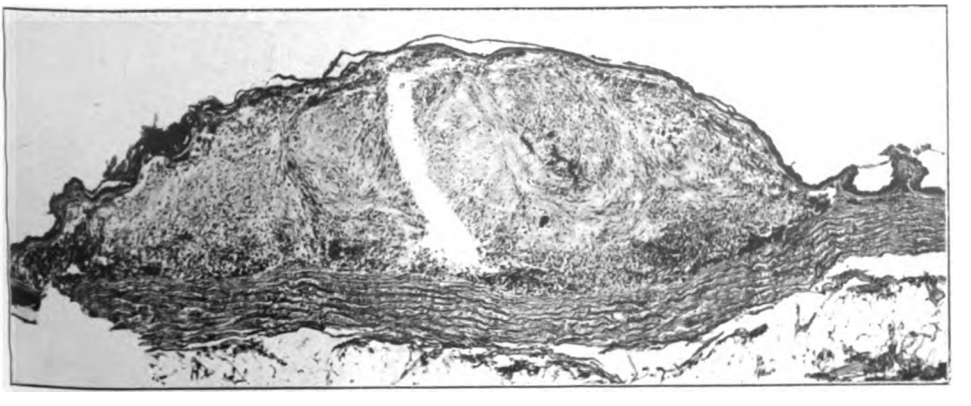


FIG. 3.

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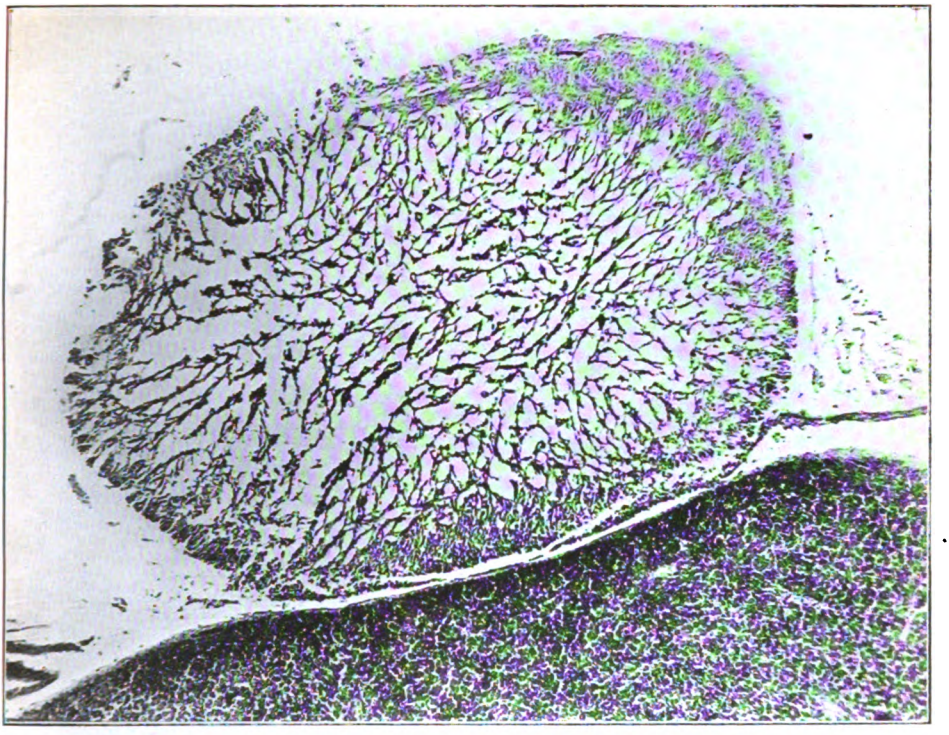


FIG. 4.

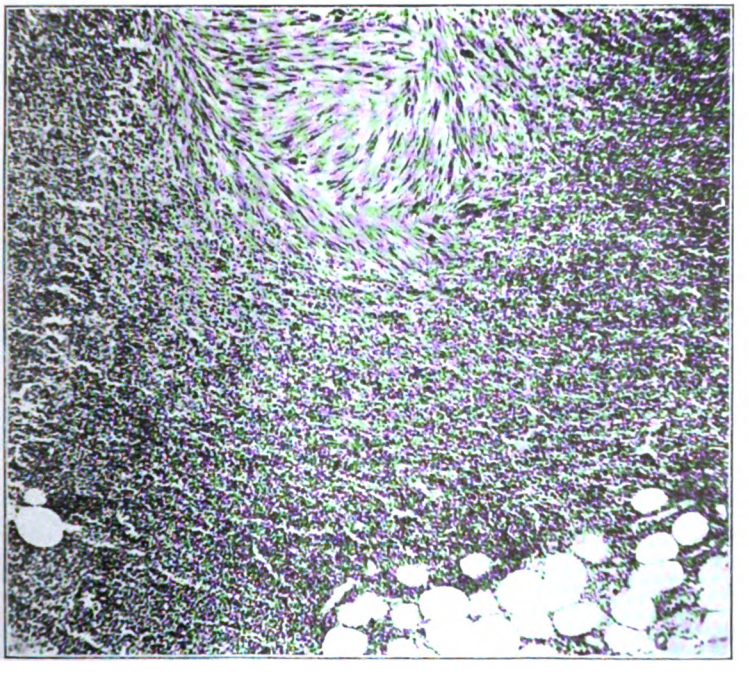


FIG. 5.

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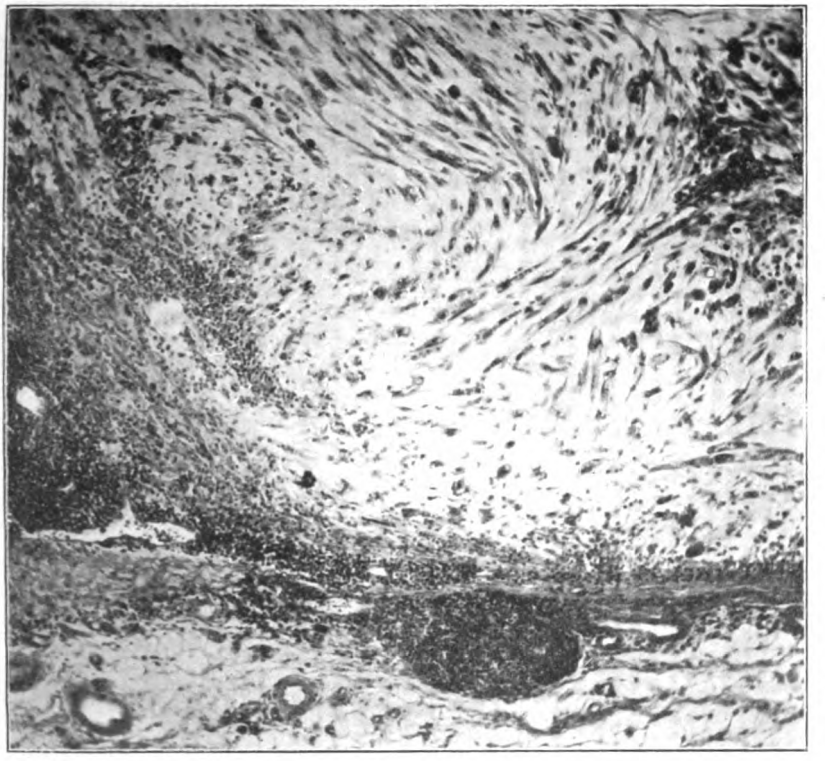
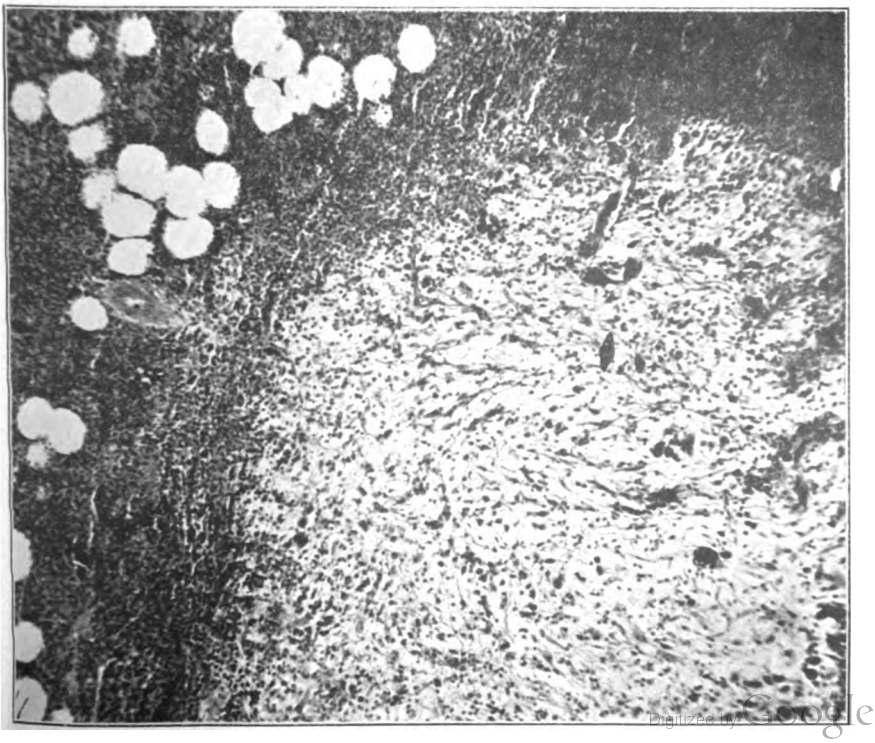


FIG. 6.



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THE PRODUCTION OF ANTIBODIES BY TISSUES LIVING OUTSIDE OF THE ORGANISM.*

BY ALEXIS CARREL, M.D., AND RAGNVALD INGEBRIGTSEN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATE 39.

INTRODUCTION.

The recent development of an efficient technique for large cultures has rendered possible the study of the functions of tissues living outside of the organism. In order to ascertain whether tissues cultivated *in vitro* retained the property of reacting against antigens by producing antibodies, we attempted to induce guinea pig bone marrow and lymph glands to become hemolytic for goat red blood corpuscles. We are very much indebted to Dr. Noguchi, whose advice enabled us to obtain immediately positive results in these experiments.

METHOD.

Guinea pig bone marrow and lymph glands were cultivated in guinea pig plasma in Gabritschewski boxes. Goat blood was selected as an antigen because it is only slightly, or not at all hemolysed by guinea pig serum. The cultures containing goat blood and their controls were incubated for five days. Then the presence of hemolysins was determined in the fluid exuded or extracted from the medium.

Preparation of the Cultures.—Blood was taken from the carotid artery of a guinea pig through an oiled cannula, and plasma was obtained by the ordinary technique.[1] The lymph glands of the neck were extirpated. Then the femurs were resected and the bone marrow was removed. After having been washed in Ringer's

* Received for publication, January 25, 1912.

[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

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solution, the bone marrow and lymph glands were cut into very small fragments and suspended in Ringer's solution. They were spread on the cover of a Gabritschewski box, and covered by plasma. In one experiment, guinea pig serum and agar were substituted for plasma. Goat blood was taken aseptically and washed three times in Ringer's solution. Two drops of washed red blood corpuscles were generally added to a culture containing twenty drops of plasma and five or six drops of a suspension of tissues in Ringer's solution. A mixture of bone marrow and lymph gland, and, exceptionally, bone marrow alone, was used. Larger and smaller quantities of blood were also employed, but the results were less satisfactory. Together with every culture containing goat red blood corpuscles, a control culture without antigen was prepared. Other control cultures were made composed only of guinea pig plasma, goat blood, and bone marrow killed by heat. When more precise microscopical examination was needed, small hanging drop cultures in hollow slides were also studied.

The cultures were deposited in the incubator at a temperature of 39° C., and examined every day from the standpoint of growth of tissues, of phagocytosis, and of possible development of bacterial colonies. After four, and generally five days, the cultures were removed from the incubator.

Examination of the Results.—The Gabritscheswki boxes were opened and the plasmatic jelly was cut in small pieces and aspirated into a large pipette. The fluid that covered the bottom of the box was also taken. Fluid and medium containing the tissues were placed in glass tubes, frozen in ice and salt, brought back to room temperature, and centrifugated. The amount of fluid extracted from each culture was 0.5 or 0.75 of a cubic centimeter. The technique of Epstein and Ottenberg,[2] which permits testing the hemolytic power of very small amounts of serum, was followed. A 5 per cent. suspension of goat washed red blood corpuscles was used. Two parts of extract were mixed with one part of a suspension of blood corpuscles. The characters of the hemolysins were investigated by the ordinary methods.

[2] Ottenberg and Epstein, *Arch. Int. Med.*, 1909, iii, 467.

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EXPERIMENTS.

Seventeen experiments were made. In several cases, complications occurred, such as premature death of the tissues, concentration of the plasma, bacterial infection, etc. These experiments were discarded, even if the results were only slightly modified. We shall describe only the experiments of which the technique was entirely satisfactory.

Experiment 1.—A culture was made, in a Gabritscheswki box, of guinea pig bone marrow and lymph gland in ten drops of guinea pig plasma and two drops of washed goat corpuscles.

The control culture contained the same amount of bone marrow and lymph gland in ten drops of guinea pig plasma, without goat blood. After four days in the incubator at 39° C., the cultures were still living and both were in the same condition. They were cut in small pieces, squeezed, frozen for ten minutes, and centrifugated. The fluid was tested against goat blood.

After two hours in the incubator (39° C.) and four hours in the ice box, there was slight hemolysis in the extract from the culture with goat blood, and no hemolysis in the control.

Experiment 2.—A culture was prepared of guinea pig bone marrow in a medium consisting of sixteen drops of guinea pig serum and four drops of 2 per cent. agar. Two drops of washed goat corpuscles were added.

The control culture contained no goat blood. After four days the cells of the cultures were still in good condition. On the morning of the fifth day in the incubator, the cultures were partially dying. They were cut in pieces, frozen, and centrifugated. The fluid was tested against goat corpuscles.

After two hours in the incubator (39° C.) and ten hours in the ice chest, the extract from the culture with goat blood showed marked hemolysis; the control showed no hemolysis.

Experiment 3.—1. A culture was prepared of guinea pig bone marrow and lymph gland in twenty drops of guinea pig plasma and two drops of goat red blood corpuscles.

2. A culture of bone marrow and lymph gland in plasma without goat blood.

3. Plasma and goat blood without tissue.

After five days the cultures were in good condition. There was no disintegration of the leucocytes. They were put in cold storage for two days and then cut in small pieces, frozen, centrifugated, and tested against goat blood. There was hemolysis in the extract from the culture with goat blood, and no hemolysis in the controls.

Then the rest of the tissue extract from the first culture (hemolysis +) was divided into two parts (I and II).

Part I was heated to 56° C. for half an hour and divided into two parts. To one of these (a), goat corpuscles were added. After two hours in the incubator and four hours in the ice chest, there was no hemolysis. The second part (b) was kept for later use.

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Part II consisted of fresh extract placed with normal goat corpuscles for four hours in the ice chest and then centrifugated for one minute. To the centrifugated cells was added normal guinea pig serum (complement); after two hours in the incubator and eighteen hours in the ice chest, there was complete hemolysis.

The control contained normal goat corpuscles and complement. There was no hemolysis. The centrifugated fluid was divided into two parts (a′ and b′). The first part (II a′) was mixed with normal goat corpuscles. After two hours in the incubator and eighteen hours in the ice chest, there was slight hemolysis. To the second part (II b′) were added equal parts of heated extract (I b) and normal goat corpuscles. There was slight hemolysis.

Experiment 4.—1. A culture was made of guinea pig bone marrow and lymph gland in twenty drops of guinea pig plasma and two drops of goat blood.

2. Control, without goat blood.

After five days in the incubator, the cultures were in good condition. They were cut in small pieces, frozen, centrifugated, and tested against goat blood. The fluid extracted from the culture with goat blood showed complete hemolysis after six to seven minutes. In the control, traces of hemolysis occurred after two hours in the incubator and eighteen hours in the ice chest.

The extract of culture 1 (hemolysis +) was divided into two parts (I and II). Part I was heated to 56° C. for half an hour, and divided into three parts. To one of these (a), goat corpuscles were added. After two hours in the incubator and eighteen hours in the ice chest, a trace of hemolysis occurred. To the second (b) were added goat corpuscles and guinea pig serum (complement). Marked hemolysis occurred after two hours in the incubator, and eighteen hours in the ice chest. The third part (c) was kept for later use.

Part II consisted of fresh extract and was kept with normal goat corpuscles for eighteen hours in the ice chest. To the sedimented corpuscles was added normal guinea pig serum (complement). After two hours in the incubator and sixteen hours in the ice chest, there was marked hemolysis.

The control contained goat corpuscles with complement. After two hours in the incubator and sixteen hours in the ice chest, there was a trace of hemolysis.

The supernatant fluid was divided into two parts, a′ and b′. The first part (II a′) was mixed with normal goat corpuscles and placed for two hours in the incubator and eighteen hours in the ice chest. There was a trace of hemolysis.

To the second part (II b′) were added equal parts of heated extract (I c) and normal goat corpuscles. After two hours in the incubator and eighteen hours in the ice chest, there was marked hemolysis.

RESULTS.

The fluid of the cultures containing goat blood has acquired the power to hemolyse markedly goat red blood corpuscles, while the serum of the control cultures remained non-hemolytic. It is certain then that hemolysins appeared in the cultures under the influence of the antigen.

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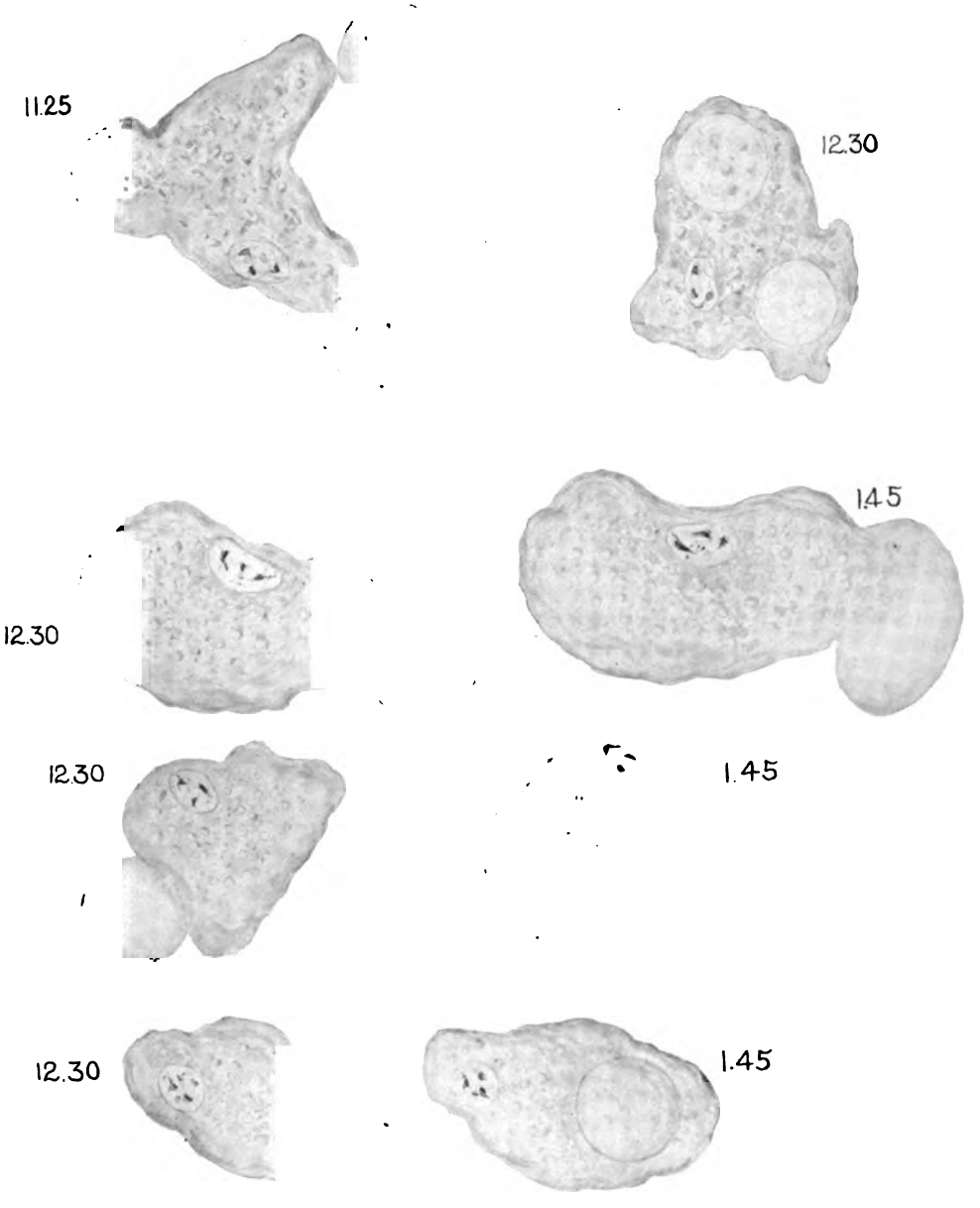


FIG. 1.

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A four day old culture already contained hemolysin, but much less than the cultures five days old. The appearance of the hemolysins was preceded by phagocytosis of the goat red blood corpuscles by the guinea pig leucocytes. Examination of the small hanging drop cultures, prepared together with the cultures in Gabritscheswki boxes, showed that, on the second day of cultivation *in vitro,* leucocytes were still inactive against the red blood corpuscles, but that on the third day they phagocyted them rapidly. Camera lucida drawings of the successive phases of phagocytosis were taken (figure 1).

The nature of the hemolytic power of the cultures acquired by the serum was then investigated. This fluid hemolysed goat red blood corpuscles without the addition of complement. After having been heated at 56° C. for half an hour, it lost its hemolytic power. Then, by addition of complement, it regained the power to hemolyse goat red blood corpuscles.

Goat red blood corpuscles were placed for four hours at 0° C. in the fluid extracted from a five day old culture. They were then separated by centrifugation, and a little complement was added. They became hemolysed, while fresh goat blood corpuscles, to which guinea pig serum was added, remained unaltered. It was found also that the fluid, separated from the red corpuscles by centrifugation and mixed with goat fresh red corpuscles, had lost almost completely its hemolytic power. Therefore, it appeared that the hemolytic power acquired by the cultures was due to substances acting as natural hemolysins.

CONCLUSIONS.

Since guinea pig bone marrow and lymph gland cultivated for five days with goat blood generate substances that are hemolytic for goat red blood corpuscles, it can be concluded that tissues living outside of the organism react against an antigen by the production of an antibody.

EXPLANATION OF PLATE 39.

The drawing represents goat erythrocytes phagocyted by guinea pig leucocytes. The corresponding figures on the left and right sides were drawn at two different times on the third day.

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A STUDY OF THE CEREBROSPINAL FLUID AND BLOOD IN ACUTE POLIOMYELITIS

GEORGE DRAPER, M.D.
AND
FRANCIS W. PEABODY, M.D.
NEW YORK

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THE CEREBROSPINAL FLUID

There are two ways in which a careful study of the cerebrospinal fluid in acute poliomyelitis may help to throw light on the disease. On the one hand, the changes observed may facilitate early diagnosis and thus enhance the value of any method of treatment which may be discovered in the future, and on the other hand, the variations in the character of the fluid, as observed during the progress of the disease in each case, may give some information as to the usual course of the process and thus be an aid in determining how far any given remedy is effective or to what extent the natural course of the disease may be influenced. The following study was thus made with the twofold object of determining whether the examination of the spinal fluid may be an aid in early diagnosis, and what changes occur in the fluid during the course of the disease.

Previous investigations along this line have not been very extensive. Most writers report only a few cases, and those who have apparently made a large number of lumbar punctures have not submitted the fluids to very careful examination. The results obtained are somewhat variable, but in general the fluid is said to contain an abnormal amount of albumin and a sediment consisting of lymphocytes, large mononuclear cells and occasionally polymorphonuclear cells. Important work has been done by Gay and Lucas,[1] whose contributions are the most valuable in this field. Their studies have embraced the spinal fluids from monkeys in the incubation period, the prodromal stage and the acute stage, and from eleven human cases in the preparalytic stage. Besides noting the character of the cells, the number of cells per cubic millimeter has been estimated. In the eleven early cases the cell count varied from fifty-five to 580 per cubic millimeter and the percentages of mononuclears from seventy-five to 100. Chemical examinations were omitted. Sophian[2] reports that the globulin test is positive in the early stages.

*This paper will form part of a monograph on the clinical aspects of acute poliomyelitis to be published by the Rockefeller Institute for Medical Research.

*From the Hospital of the Rockefeller Institute for Medical Research, New York.

1. Gay and Lucas: Arch. Int. Med., 1910, vi, 330; Lucas: AM. JOUR. DIS. CHILD., 1911, i, 230.

2. Sophian: Arch. Pediat., March, 1911.

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Inasmuch as previous work by Wollstein,[3] Römer and Joseph,[4] and Gay and Lucas[1] failed to demonstrate the presence of any specific antibody in the spinal fluid in acute poliomyelitis, we have confined our study to simple chemical and cytological examinations.

It has been our object to see the cases as early as possible after the onset of the disease, to make a lumbar puncture on admission to the hospital, to repeat the puncture every two to four days in the early stages, and in the later stages to make a puncture every five to ten days while the case was under observation, or until the fluid became normal. We have examined in all 233 fluids from sixty-nine cases. The number of cases seen in the first week of the disease (dating from onset of symptoms, not of paralysis), was forty-three, in the second week forty-five, in the third week forty, and in the fourth week thirty. The number of cases seen later in the course was much smaller. It is to be regretted that the number of preparalytic and abortive cases was so small — of the former, four, and of the latter, only two were seen in the active stage. It was, however, rare that cases were referred to the hospital until a positive diagnosis, based on the appearance of paralysis, had been made by the attending physician.

The methods used for the examination of the cerebrospinal fluid have included estimation of the pressure, determination of the number of cells per cubic millimeter and the types of cells present, estimation of the chlorids and observation of the power of reducing Fehling's solution, and on the presence of globulin. Globulin was tested for by the butyric-acid method of Noguchi and the relative strength of the reaction noted. Thus "very slightly plus" (v. s. +) indicates a faint precipitate, which is, however, definitely more than that obtained with normal spinal fluids; "slightly plus" (s. +) is the term applied to reactions giving a well-marked cloud or a slightly flocculent precipitate; "plus" (+) signifies a precipitate coming down in large flocculi; and "double plus" (+ +) a heavy flocculent precipitate. The reaction was performed with accurately measured amounts of spinal fluid and of reagents, and the reading made after five to ten minutes' standing.[5] The gross appearance of the fluids was very constant. Almost all were clear, colorless and watery. A few, only, showed the slightest opalescence. On standing, a delicate web-like clot formed in a small proportion of cases. Clot formation was most frequently seen in the early stages of the disease. It bore no apparent relation to the globulin content of the fluid.

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3. Wollstein: Jour. Exper. Med., 1908, x, 476.

4. Römer and Joseph: München. med. Wchnschr., 1910, lvii, 568.

5. To 0.2 c.c. spinal fluid is added 0.5 c.c. butyric acid solution (10 per cent. butyric acid in 0.85 per cent. solution of sodium chlorid). The mixture is boiled, 0.1 c.c. normal sodium hydrate solution added, and then boiled again.

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The results obtained by several of these methods may be dismissed briefly. Pressure determinations were made in a number of cases and the readings were usually above normal. The pressure was read in milimeters of spinal fluid in a tube of 1.0 mm. bore. No extremely high readings were obtained, and in some instances normal pressures were seen during the early stages of the disease. On the whole, the estimations of pressure were unsatisfactory, as the patients were usually crying children.

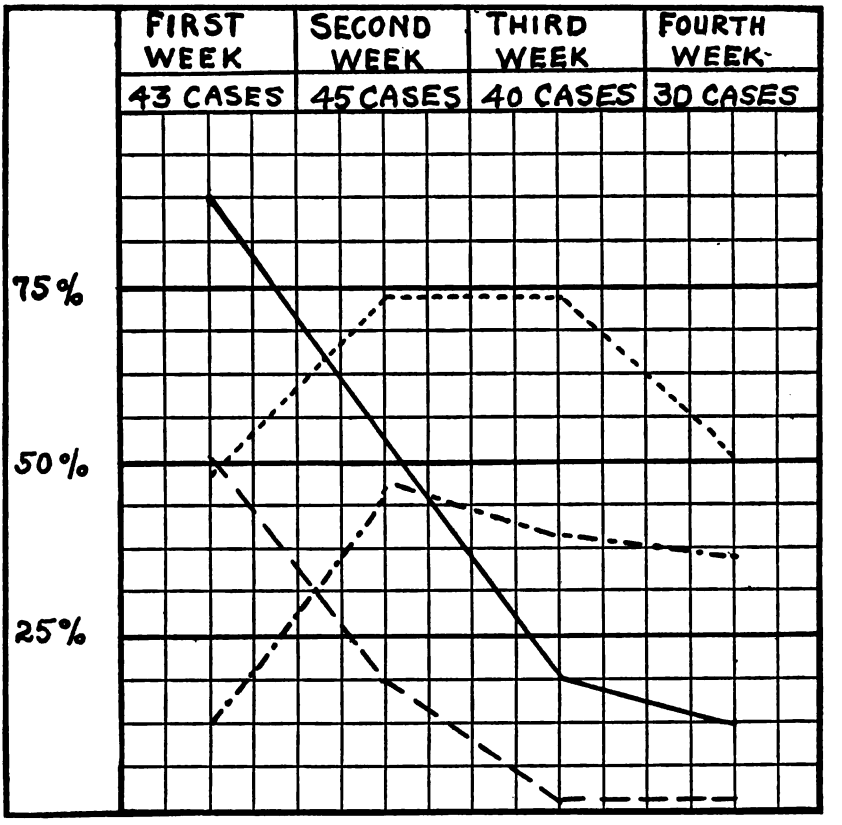


Chart showing variations in cell-count and in globulin content of cerebrospinal fluid in infantile paralysis. ——— Percentage of cases with cell-count above normal. — — — Percentage of cases with cell-count above 50 per c.mm. - - - - Percentage of cases with globulin "s+". —.—.—. Percentage of cases with globulin "+" or over.

The chlorids were estimated in a series of cases with the idea that they might throw light on the inorganic substances in the fluid. No marked deviations from the normal were observed. Every fluid examined showed a power of reducing Fehling's solution which was, roughly speaking, normal. This reaction serves as a point of differentiation from many

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fluids in cases of meningitis, in which the reducing substance may be absent.

The type of cell present in the fluid was almost always the mononuclear. A large number of differential counts showed that the relative number of the different types of mononuclear cells was of comparatively little importance, so in later observations we were content with merely noting the relative number of mononuclear and polymorphonuclear cells. In several instances, numbers of very large phagocytic cells containing vacuoles and broken-down cells were seen. Once this occurred several weeks after the onset of the disease, in association with a sudden rise in the cell count resembling that seen in acute meningitis. Polymorphonuclear cells, except in small number and probably depending for the most part on a slight admixture of blood, were, except in a very few instances, absent after the first week of the disease. Early in the disease, however, and in the preparalytic period, the polymorphonuclears sometimes outnumbered the mononuclears and made up 80 to 90 per cent. of the total.

The results described by Flexner and Lewis[6] of a study of the cerebrospinal fluid of a monkey after inoculation with the virus of poliomyelitis are interesting. Twenty-four hours after inoculation the fluid contained "a considerable number of small cells, hardly exceeding a lymphocyte in size but showing a polyform nucleus, a few lymphocytes and some red corpuscles. At the expiration of forty-eight hours, the white cells have increased in numbers, but the cells with polyform nuclei still predominate. At the expiration of seventy-two hours, a large number of mononuclear cells have appeared and the fluid presents a striking opalescent appearance. On the day of paralysis the fluid tends to be only slightly cloudy and contains a mixture of large and smaller (lymphoid) mononuclear cells and a few cells with polymorphous nuclei." Lucas, too, found in the monkey during the prodromal stage "a marked increase in the cells, often reaching 1,000 per cubic millimeter. In this stage, also, polynuclears are still present, in some cases as high as 60 per cent., though the large mononuclears and lymphocytes were very evident. In the early acute stage the increase in cells is very marked. The cells are now, however, mostly of the lymphocytic or very early form of cells, and sometimes very hard to place, as they are apparently undifferentiated cells." Of his eleven preparalytic human cases, however, only one showed less than 87 per cent. mononuclears, and that had 75 per cent. mononuclears. A return of polymorphonuclears later in the disease, as was reported by Lucas as occurring in monkeys, was not noticeable in our human cases.

The cell count was made immediately after the fluid was obtained. The first portion of fluid obtained was used, but controls made by com-

6. Flexner and Lewis: Jour. Am. Med. Assn., 1910, liv, 1140.

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paring counts of early and late portions of the fluid showed no important discrepancy. While the normal number of cells per cubic millimeter of spinal fluid is usually given as three to five, we have considered the upper normal limit as ten to twelve. In general it may be said that the highest cell counts are found in the early days of the disease, and that there is a progressive falling off as time advances. Thus of forty-three cases in the first week, twenty-three showed fluids with over fifty cells per cubic millimeter and thirty-eight with cell counts above normal. Two cases showed in the prodromal period 990 and 650 cells, respectively. Of forty-five cases in the second week, in eight the counts were over fifty, and in twenty-three cases they were above normal; of forty cases in the third week, in only one was the count over fifty, and only in eight above normal. On the other hand, six cases in the first week, twenty-two cases in the second week and thirty-two of the forty cases in the third week showed normal counts.

In contrast to the cell count which thus tends to be high in the early part of the disease, the globulin content is usually low in the first part of the acute stage, especially as compared with the cell count. It rises during the second and third weeks and then gradually falls, though frequently globulin is present long after all acute symptoms have passed (see chart). We have seen a strong (+) globulin reaction on the forty-sixth day of the disease. During the first week, only six of forty-three cases showed at any time a globulin reaction of + (see above) or more. During the second week twenty out of forty-five showed a + reaction, in the third week sixteen out of forty, and in the fourth week nine of thirty cases. On the other hand, sixteen cases in the first week showed an absolutely normal globulin reaction. Five cases in the second week and only three in the third week were negative for globulin. Most cases, even on discharge, gave a globulin reaction which varied from slightly to markedly above the normal. In eleven cases the fluids were followed until both cell count and globulin reaction became normal. The return to normal occurred in the third week in two cases, in the fourth in two cases, in the fifth in one case, in the sixth in two cases, in the seventh in three cases, and in the tenth week in one case.

Of the sixty-nine cases studied, only two never showed any abnormal fluids. In each of these cases the fluid was examined but once, on the twenty-fifth and thirty-third days of the disease, respectively. One case showed, in five fluids taken between the ninth and forty-first days of the disease, no evidence of abnormality other than the slightest positive globulin reaction (v. s. +), and another, in three fluids obtained on the ninth, sixteenth and twenty-third days, gave similar results. Four cases, including one abortive case, showed fluids which, except for the slightest

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globulin reaction, were normal at the first tapping and subsequently became more definitely positive.

The commonest type of fluid seen in the whole series is one with normal or slightly increased cell count and a well-marked globulin reaction. There are, however, two other distinct types of fluid met with. The one is a fluid with a high cell count and a normal or very slight globulin reaction, and the other is a fluid with a normal or low cell count and a very marked globulin reaction. Twenty-six cases showed at some time a globulin of not over s. + and cell count of fifty or over. Twenty of these occurred in the first week, and in twelve the count was over 100 cells per cubic millimeter. On the other hand, sixty-two fluids from thirty-four cases gave a globulin reaction of + or more. In thirty-three of these the cell count was normal and in fifty-five it was not above forty per cubic millimeter. That the two types of fluid are fairly distinct is shown by the fact that only five cases showed fluids with both a high cell count (over fifty), and even a moderately high globulin. Each type of fluid is, moreover, quite definitely characteristic of a stage in the disease. The cellular exudate is almost always associated with the earliest days of the acute stage, the albuminous exudate with the latter part of the acute stage. It is interesting that of ten cases seen within the first three days after the onset of symptoms, all but one had a cell count of over fifty (one was thirty-seven), and six were over 100. On the other hand, six gave a negative globulin reaction. In four the reaction was s. +.

DIAGNOSIS

As regards early diagnosis, the cases examined before the onset of paralysis are of the greatest interest. Six cases were seen in what may be termed the prodromal period. Four of these developed paralysis later and two proved to be of the abortive type. In one case a single lumbar puncture was made on the fourth day after the onset. The fluid gave a cell count of 650 per cubic millimeter, 90 per cent. polynuclears, and a + globulin reaction. Three days later the child suddenly developed paralysis and in five hours died of respiratory failure. A second case, the sister of a child already in the hospital, was seen on the day after the onset of symptoms. The spinal fluid contained 990 cells per cubic millimeter, 90 per cent. of which were polymorphonuclears, and the globulin reaction was s. +. On the following day one leg was paralyzed and the cell count of the spinal fluid was 627 per cubic millimeter with 80 per cent. mononuclears. The next day both legs were paralyzed and the cell count was 1,221 per cubic millimeter, with 92 per cent. mononuclears including many large phagocytic cells. The globulin reaction was +. Thus two cases seen very early in the course of the disease showed fluids with unusually high cell counts, and with a marked pre-

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dominance of polymorphonuclear cells. In one of them the change of the cell picture from the polynuclear type to the mononuclear type could be followed coincident with the onset of the paralysis. Three other cases in the prodromal period, one an abortive case and two becoming paralyzed on the day of the first lumbar puncture, gave cell counts of thirty-seven, ninety-four and 113 per cubic millimeter, with 100 per cent., 93 per cent. and 83 per cent. of mononuclear cells, respectively, and globulin reactions which fell within normal limits. Finally in one abortive case lumbar puncture on the second day after the onset of symptoms gave a wholly normal fluid. On the third day the cell count was sixty-two per cubic millimeter, with 89 per cent. mononuclears and a s. + globulin reaction. On the sixth day the cell count was fifty-nine per cubic millimeter, all mononuclears, and globulin s. +. On the eleventh day the spinal fluid was again normal. That this was in fact an abortive case of poliomyelitis was proved later by showing that the patient's blood-serum was capable of neutralizing active virus when mixed with it and injected intracranially into a monkey.

In these six cases, then, examination of the spinal fluid gave evidence which was helpful in making a diagnosis of poliomyelitis before the onset of paralysis. Similar evidence was afforded by eleven cases of Lucas and by one of Frissell.[7] In none of these twelve cases, however, was a large percentage of polynuclear cells found. But it seems of importance to call attention to the fact that a fluid whose cytology resembles that found in cerebrospinal meningitis and other types of purulent meningitis may also occur in the early stages of poliomyelitis.

The question arises as to whether there is any relation between the situation and extent of the paralysis or the severity of the disease and the character of the spinal fluid. Conclusions on this point cannot be drawn from the number of cases seen by us. It would seem, however, that in cases in which the paralysis is limited to arms or to cranial nerves, the lumbar puncture fluid is rather apt to show less deviation from the normal than where the legs are involved. This is, however, far from being a constant finding. Several cases which had acute symptoms (pain, irritability), lasting for an unusually prolonged period, gave an unusually large and persistent globulin reaction. Moreover, the disappearance of the acute symptoms was frequently coincident with a diminution in the globulin in the spinal fluid. The fatal cases showed nothing in the fluid on which to base a bad prognosis.

Since the infecting organism in poliomyelitis is too small to admit of its being seen, even if it is present in the cerebrospinal fluid, and since the more complicated biological tests have failed to prove the presence of antibody in the fluid, the diagnostic value of examinations of the

7. Frissell: Jour. Am. Med. Assn., 1911, lvi, 661.

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spinal fluid by simple chemical and microscopic methods must necessarily be merely relative. While in cerebrospinal meningitis, in tuberculous meningitis, in pneumococcus, influenzal and similar types of meningitis one obtains specific information and is usually enabled to make a positive diagnosis from an examination of the spinal fluid, the value of the examination in poliomyelitis is necessarily less direct. Thus in a fluid containing a high percentage of polymorphonuclear cells, the failure to find any organisms would certainly be suggestive of poliomyelitis. The difficulty of differential diagnosis is perhaps greater when one has a fluid with a lymphocytic cytology. Lucas has discussed at length the non-specificity of the cytological findings in various meningeal conditions, and finds a similar cell picture in encephalitis, poliomyelitis, meningismus, tuberculous meningitis and syphilis of the central nervous system. Because, however, the examination of the spinal fluid in poliomyelitis lacks specific value, it would be very wrong to conclude that it was not an important aid in diagnosis, or that lumbar puncture should not be performed in suspicious cases. Routine blood examinations and the usual urinary analyses have comparatively rarely any specific diagnostic value. Their greatest usefulness consists, perhaps, in helping to rule out certain possibilities, and in focusing one's attention on a narrower group of processes. Within such a group the blood or urine examination, partly of itself, and partly when brought into line with clinical experience, may, by bringing either negative or positive evidence, be the determining factor which inclines one toward the correct diagnosis. The value of positive findings in spinal fluid examinations is undoubted. The value of negative results in examination approaches it in importance. Thus in the case of poliomyelitis, the failure to demonstrate specific changes in the spinal fluid should rule out several serious meningeal conditions, the differential diagnosis of which from poliomyelitis in the preparalytic stage may be most confusing. On the other hand, by the demonstration of non-specific changes (for we have seen that by far the greater number of fluids in poliomyelitis are in one way or another abnormal), a host of other conditions which may simulate incipient poliomyelitis are removed from further consideration. Among the small group of remaining possibilities, certain characteristic types of fluid, as for instance one with a high cell count and a normal globulin reaction, may be of some value in deciding the diagnosis. More careful studies of the fluids in conditions other than poliomyelitis may throw further light on the differential value of non-specific fluids. At any rate, when even the non-specific fluid of poliomyelitis is considered in association with the clinical features of the case in hand, the diagnosis can probably be made in the great majority of cases even in the preparalytic stage. The ease and safety with which lumbar puncture can be performed and

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the simplicity of the examination of spinal fluids should make the method one of much wider use than it is at present. The value of any future method of treatment of poliomyelitis must depend eventually on the possibility of early diagnosis, for where nerve cells have been destroyed, the results from any therapeutic measures will be comparatively small.

CONCLUSIONS

The spinal fluid from cases of acute poliomyelitis during the first few weeks after the onset of symptoms shows, in the great majority of instances, deviations from the normal.

Fluids taken during the early days of the disease, and especially before the onset of paralysis, tend to show an increased cell count with a low or normal globulin content.

At this early stage the polymorphonuclear cells may amount to 90 per cent. of the total. Most fluids, however, show lymphocytes and large mononuclear cells almost exclusively.

After the first two weeks the cell count usually drops to normal, or nearly to normal, and there is frequently an increase in the globulin content. A slight increase in globulin may persist for seven weeks or more.

Analogous changes may be found in the spinal fluid of abortive cases.

All fluids examined reduced Fehling's solution.

The examination of the cerebrospinal fluid in acute poliomyelitis, while giving, as far as is yet known, no specific diagnostic criteria, is of the utmost value as an aid to diagnosis both in preparalytic and in abortive cases.

THE BLOOD

The complete symptomatology of diseases caused by filterable virus in man is not yet established. Certain clinical features of the two most studied examples, hydrophobia and poliomyelitis, have been well described, but the blood picture which has been so thoroughly determined in most infectious diseases and is of such value in differential diagnosis, is little discussed. Owing to the resemblance of numerous features of rabies and poliomyelitis, it seemed that a comparative study of the blood changes in the two diseases might throw some further light on the nature of the human body's reaction to invasion by infecting agents of this type. The large systems of medicine make no mention of blood counts in either malady. In one exhaustive report of thirty cases of rabies by Brain and Maloney the blood of one patient is reported to have had a "slight polymorphonuclear leukocytosis."

Through the courtesy of the attending physicians at the Presbyterian Hospital, in New York, the following blood counts from cases of hydrophobia are available:

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Patient 1.—Male, aged 35; temperature, 99.3 F.; W. B. C., 9,400; polymorphonuclears, 76.0 per cent.; lymphocytes, 10.5 per cent.; large mononuclears, 9.8 per cent.; transitionals, 3.8 per cent.; basophils, 0.0 per cent.; eosinophils, 0.0 per cent.

Patient 2.—Male, aged 27; temperature, 100 to 103 F.; W. B. C., 13,000: Polymorphonuclears, 76.5 per cent.; lymphocytes, 11.0 per cent.; large mononuclears, 10.0 per cent.; transitionals, 2.5 per cent.; basophils, 0.0 per cent.; eosinophils, 0.0 per cent.

Patient 3.—Male, aged 27; temperature, 103 F. (Nov. 26). W. B. C., 34,450: Polymorphonuclears, 85.0 per cent.; lymphocytes, 12.0; large mononuclears, 0.0 per cent.; transitionals, 0.3 per cent.; basophils, 0.0 per cent.; eosinophils, 0.0 per cent.

Patient 3.—Temperature 103 F. (Nov. 27). W. B. C., 30,200: Polymorphonuclears, 87.0 per cent.; lymphocytes, 10.5 per cent.; large mononuclears, 0.5 per cent.; transitionals, 2.0 per cent.; basophils, 0.0 per cent.; eosinophils, 0.0 per cent.

Obviously, except in the first case, in which the total count was normal, there was a leukocytosis. In all the cases a definite polynucleosis existed with low lymphocyte percentage.

In the literature of poliomyelitis there are conflicting statements about the leukocyte count. LaFetra[8] in New York and Müller[9] in Germany have made the most extensive observations on the blood. The former reported in six cases a leukocytosis, running from 13,400 to 20,600. He says nothing about the differential counts. Müller, on the other hand, reporting fifteen cases, says that a leukopenia of from 3,000 to 5,000 was always present in the acute stage. He gives no figures but states that there was a slight increase of the lymphocytes. Gay and Lucas[10] made a comparative study of the blood in monkeys suffering from the experimentally produced disease and in children with poliomyelitis. In monkeys they report a leukopenia during the acute stage with a lymphocytosis ranging from 10 to 20 per cent. above normal. The protocols of the human cases, however, do not offer very convincing support to their statements that a similar blood picture occurs in the human cases. The lowest count reported from a 9 months' baby (Case 2), is 7,800 to 12,200. The other three cases showed the following leukocyte counts:

Case 1, 20 months, 11,600 to 17,400.
Case 3, 2 years, 12,000.
Case 4, 5 years, 13,400.

These blood counts can hardly be considered below normal. In two of their four cases a lymphocytosis, "slight" for one and "62 per cent." for the other, is given. Of the other two cases, one, a child of 5 years, had a normal differential count and the other a polynucleosis of 75.5 per cent.

The accompanying tables and ensuing statements are based on the study of the blood of our seventy-one hospital patients. The actual figures given represent the counts from only fifty-nine cases. The records

8. La Fetra: Arch. Pediat., 1909, xxvi, 328.
9. Müller: Die Spinale Kinderlähmung, Berlin, 1910.
10. Gay and Lucas: Arch. Int. Med., 1910, vi, 330.

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of the other twelve cases were omitted because those patients suffered from other conditions which might have affected the blood count. Care was taken to avoid collecting the blood at times when a digestive leukocytosis might have been present. The total white count was made in the usual way, the blood and 0.5 acetic acid being mixed in a 1-10 pipette and the drop counted on a 0.1 mm. cell. For differential counts Wright's modification of the Romanowski stain was used.

TABLE 1.—DIFFERENTIAL BLOOD-COUNT IN CASES OF ACUTE POLIOMYELITIS

ge, ears	Number of cases			Poly.	Lymph.	L.Mono.	Trans.	Baso.	Eosin.	Stim.*
			First Week							
-1	4	23,900-35,600	Max.	59.0	57.0	18.0	15.0	0.5	1.0	1.5
			Min.	25.0	16.5	1.0	0.5	0.0	0.0	0.0
			Aver.	42.0	36.7	9.5	7.7	....	....	....
-2	12	12,200-26,000	Max.	70.0	50.0	13.5	13.5	1.0	15.5	1.0
			Min.	26.5	16.5	1.5	2.5	0.0	0.0	0.0
			Aver.	48.2	33.2	7.5	8.0	....	....	....
-3	5	9,000-23,000	Max.	64.5	43.0	14.0	18.0	1.5	7.0	1.5
			Min.	32.5	20.0	1.5	3.0	0.0	0.0	0.0
			Aver.	48.5	31.5	7.7	10.5	....	....	....
-4	4	6,900-15,000	Max.	72.0	29.0	8.5	13.0	0.5	9.0	1.0
			Min.	55.0	17.0	1.0	1.5	0.0	0.0	0.0
			Aver.	68.5	23.0	4.7	7.2	....	....	....
-5	6	12,400-33,000	Max.	81.0	37.5	26.5	14.5	1.5	0.0	0.5
			Min.	44.5	18.6	2.6	4.5	0.0	0.0	0.0
			Aver.	62.7	28.0	14.5	9.5	....	....	....
-7	4	8,800-23,300	Max.	77.0	24.0	11.7	5.5	1.0	8.0	1.0
			Min.	62.2	16.7	0.5	1.0	0.0	0.0	0.0
			Aver.	69.6	20.3	6.5	3.2	....	....	....
-21	3	12,000-16,700	Max.	75.0	17.5	17.0	9.5	0.05	1.5	0.0
	—		Min.	59.0	13.0	0.5	4.5	0.0	0.0	0.0
	38		Aver.	67.0	15.2	8.7	7.0	....	....	....
			Second Week.							
-2	11	12,300-30,400	Max.	73.0	56.0	23.0	11.0	6.0	5.5	0.05
			Min.	37.0	9.0	0.5	4.0	0.0	0.0	0.0
			Aver.	55.0	32.5	11.7	7.7	....	....	....
-3	8	9,800-20,000	Max.	61.5	41.0	23.5	10.5	5.0	7.5	0.5
			Min.	38.0	10.0	2.0	2.0	0.0	0.0	0.0
			Aver.	49.7	25.5	12.7	6.2	....	....	....
-4	2	14,600-19,700	Max.	84.0	21.5	13.5	6.5	0.0	0.5	0.5
			Min.	57.5	6.5	6.5	3.0	0.0	0.0	0.0
			Aver.	70.7	14.0	10.0	4.7	....	....	....
-5	1	28,000								
-7	1	11,800		76.5	14.5	4.5	3.5	0.0	0.5	0.5
-12	1	12,000		62.0	17.5	16.0	3.5	0.0	1.0	0.0
	—									
	24									

*Stim="Stimulation Forms" of Türck.

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Third Week

Age, Years	Number of Cases			Poly.	Lymph.	L.Mono.	Trans.	Baso.	Eosin.	Stim.
1-2	13	8,300-26,100	Max.	67.0	58.5	7.5	13.5	1.5	10.5	1.0
			Min.	30.5	22.0	0.0	0.5	0.0	0.0	0.0
			Aver.	48.7	40.2	3.7	7.0	....	....	...
2-3	5	12,800-23,700	Max.	63.5	42.5	24.5	9.5	0.5	3.0	0.5
			Min.	45.0	16.0	0.0	2.5	0.0	1.0	0.0
			Aver.	54.7	29.2	12.2	6.0	....	....	....
3-4	2	10,300-15,700	Max.	59.5	37.5	6.5	14.0	1.0	6.5	0.0
			Min.	45.0	20.0	2.0	5.5	0.0	0.5	0.0
			Aver.	52.2	28.7	4.2	9.7	....	....	...
4-5	1	28,000								
5-7	1	28,000		72.0	9.0	2.0	10.0	0.0	2.0	0.0
8-12	2	7,800-18,000	Max.	67.5	28.0	4.5	9.0	0.0	2.5	0.0
			Min.	63.0	19.5	3.5	2.0	0.0	1.0	0.0
	24		Aver.	65.2	23.7	4.0	....	....	....	....

Fourth Week

1-2	11	10,100-24,700	Max.	60.0	63.5	41.0	17.0	1.0	4.0	2.5
			Min.	26.5	21.5	35.0	1.5	0.0	0.0	0.0
			Aver.	43.2	42.5	23.7	9.5	....	....	....
2-3	5	14,600-23,600	Max.	65.0	46.0	10.5	10.0	0.5	6.0	0.0
			Min.	26.5	23.5	0.5	4.5	0.0	0.0	0.0
			Aver.	45.7	34.7	5.5	7.2	....	....	....
3-4	2	11,000-19,800	Max.	51.0	46.5	1.5	8.5	0.5	8.0	0.5
			Min.	40.5	37.5	0.0	3.5	0.0	2.0	0.0
			Aver.	45.7	42.0	0.7	6.0	....	....	....
4-5	3	10,500-22,300	Max.	58.5	33.8	10.5	4.0	0.6	2.7	0.3
			Min.	42.0	27.9	6.4	2.0	0.0	0.5	0.0
			Aver.	50.2	30.8	8.4	3.0	....	....	....
5-7	2	14,200-21,000	Max.	64.5	36.0	12.5	2.5	0.5	0.0	0.0
			Min.	49.5	25.0	7.5	1.5	0.0	0.0	0.0
			Aver.	57.0	30.0	10.0	2.0	....	....	....
8-12	2	9,400-10,700	Max.	64.5	26.0	2.5	8.0	0.5	4.5	0.5
			Min.	62.5	....	0.0	4.5	0.0	0.5	0.0
	25		Aver.	63.5	....	1.7	6.2	....	....	....

Fifth Week

1-2	6	9,100-23,600	Max.	60.5	56.0	13.5	10.0	0.5	6.5	1.0
			Minn.	38.0	20.5	1.0	2.0	0.0	1.5	0.0
			Aver.	49.2	38.2	7.2	6.0	....	....	....
2-3	6	15,000-27,000	Max.	58.0	44.5	9.5	14.0	0.0	9.5	0.5
			Min.	40.0	21.5	0.0	5.0	0.0	3.5	0.0
			Aver.	49.0	33.0	4.2	9.5	....	....	....
3-4	3	12,800-16,700	Max.	60.5	30.0	10.5	6.5	1.0	4.0	0.5
			Min.	54.5	25.0	6.0	2.5	0.0	2.0	0.0
			Aver.	57.5	27.5	8.2	4.5	....	....	....
4-5	2	6,300-20,000	Max.	85.0	24.0	12.0	7.0	1.0	2.0	1.0
			Min.	62.5	19.0	0.5	3.5	0.0	....	0.0
	17		Aver.	73.7	21.5	6.2	5.2	....	....	....

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Sixth Week

Age, Years	Number of Cases			Poly.	Lymph.	L.Mono.	Trans.	Baso.	Eosin.	Stim.
			Max.	40.0	69.0	10.0	15.0	0.5	2.5	2.0
1-2	3	9,600-26,700	Min.	23.0	32.0	0.0	0.5	0.0	0.5	0.0
			Aver.	31.5	50.5	5.0	7.7	....	....	....
			Max.	57.0	44.0	4.5	9.0	0.5	3.5	2.0
2-3	2	10,200-22,800	Min.	45.0	29.0	2.0	0.5	0.0	0.5	0.5
			Aver.	51.0	36.5	3.2	4.7	....	....	....
			Max.	64.0	34.0	8.5	3.0	0.0	1.0	0.0
4-5	2	13,100-28,600	Min.	57.5	23.5	7.0	1.5	0.0	0.0	0.0
			Aver.	60.7	28.7	7.7	2.2	....	....	....
5-7	1	15,000		50.0	32.5	4.0	11.0	0.0	1.5	1.0
8-12	1	9,400		47.0	37.5	5.5	4.0	0.0	3.5	2.5
	9									

Seventh Week

			Max.	51.0	45.0	10.0	5.0	2.0	6.5	0.5
1-2	2	23,700-26,700	Min.	31.5	34.5	9.5	4.0	0.0	0.5	0.0
			Aver.	41.2	39.7	9.7	....	....	....	....
			Max.	70.0	43.6	11.0	7.5	0.5	5.0	0.0
2-3	3	15,800-22,300	Min.	42.5	16.0	0.5	3.5	0.0	1.0	0.0
			Aver.	56.2	29.8	5.7	5.5	....	....	....
3-4	1	9,800		49.0	30.5	4.0	11.5	0.5	4.0	0.0
			Max.	62.0	54.5	10.5	4.0	9.5	1.0	0.5
4-5	2	11,000-12,400	Min.	39.5	16.0	0.5	0.5	0.5	0.5	....
			Aver.	50.7	35.2	5.5	2.2	....	....	....
5-7	1	16,000		59.5	22.0	7.5	8.5	0.5	2.0	0.0
	9									

Eighth Week

			Max.	52.5	37.5	9.0	5.5	0.0	1.5	0.0
4-5	2	10,700-12,000	Min.	47.0	35.5	8.5	2.5	0.0	0.5	....
			Aver.	49.7	36.5	8.7	4.0	....	....	....
8-12	1	16,000		58.0	32.5	2.0	6.0	0.0	2.0	0.5
	3									

Tenth Week

4-5	1	10,800		61.5	23.5	4.5	9.0	0.0	0.0	0.5

Twelfth Week

4-5	1	16,000		54.0	38.0	5.5	2.0	0.0	1.5	0.0

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The first set of tables is designed to show minimal, maximal and average counts for groups of cases. A second, composite table shows minimal, maximal and average total leukocyte counts of all ages together by weeks. Such a compilation seems justifiable because the age variation is much less in respect to the total than the differential leukocyte count.

TABLE 2.—COMPOSITE TABLE SHOWING MINIMAL, MAXIMAL AND AVERAGE TOTAL LEUKOCYTE COUNTS OF ALL AGES BY WEEKS

Week	Number of Cases	Maximal	Minimal	Average
First	38	24,600	12,200	18,400
Second	24	24,500	12,000	18,250
Third	24	23,300	10,400	16,800
Fourth	25	20,300	11,600	15,900
Fifth	17	21,800	10,800	16,300
Sixth	9	23,200	10,500	16,800
Seventh	9	19,500	15,000	17,250
Eighth	3	14,000	10,700	12,400
Tenth	1	.....	.....	10,800
Twelfth	1	.....	.....	16,000
	151			

TABLE 3.—THIS TABLE COMPRISES SEPARATELY THE COUNTS FROM PREPARALYTIC AND ABORTIVE CASES

Case	Age, Yrs.		W. B. C.	Poly.	Lymph.	L.Mono.	Trans.	Baso.	Eosin.	Stim.
B. X.	5		15,000	....	....	....	....	....	....	....
		3/0*	7,000	81.0	....	....	....	...	....	....
J. S.	2½	2/0	14,200	58.5	31.5	7.0	3.0	0.0	0.0	0.0
C. T.	⅔	2/0	35,600	32.0	55.0	1.0	9.0	0.0	1.0	1.5
E. N.	6	6/0	23,200	76.0	17.0	3.0	2.5	0.0	1.0	0.5
H. B.	6	2/0	8,800	77.0	17.5	0.5	4.0	0.0	0.0	1.0
		3/0	11,200	79.5	14.5	0.5	4.5	0.0	0.5	0.5
		6/0	5,600	68.5	22.0	1.5	6.0	0.0	1.0	1.0
		13/0	11,800	76.5	14.5	4.5	3.5	0.0	0.5	0.5
P. T.	2	1/0	15,000	52.5	31.5	13.5	1.5	0.5	0.5	0.0

*The numerator of the fraction signifies the day of the disease, the denominator the day of the paralysis.

It is obvious from these figures that while in most cases in the preparalytic stage the total leukocyte count varies within the normal, there is a tendency toward the upper limits. More cases showed counts well above than below the usual extremes. In the differential counts there is a definite polynucleosis with the one exception of an 8 months' infant in whom the relation of polymorphonuclears and lymphocytes is normal. Furthermore, the lymphocyte percentages in all the other cases are distinctly below the usual figure.

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TABLE 4.—DIFFERENTIAL COUNTS IN NORMAL INDIVIDUALS

Age, Years		Poly.	Lymph.	L.Mono.	Eosin.	Baso.
	Max.	33.9	58.5	12.2	4.5	0.8
½-1	Min.	24.6	50.5	7.3	0.0	0.1
	Aver.	30.4	55.9	9.6	2.6	0.4
	Max.	39.7	58.8	11.7	6.0	0.5
1-2	Min.	27.5	45.3	6.7	1.6	0.0
	Aver.	36.3	51.2	8.5	3.2	0.2
	Max.	44.3	55.0	11.3	6.0	1.2
2-3	Minn.	33.2	43.5	5.0	0.5	0.0
	Aver.	38.7	49.0	8.2	3.1	0.4
	Max.	54.1	47.6	16.2	4.2	0.9
3-4	Min.	36.2	32.2	5.0	1.5	0.0
	Aver.	44.7	39.1	11.2	2.8	0.5
	Max.	51.7	49.5	6.7	4.0	0.6
4-5	Min.	42.2	38.4	3.4	1.6	0.3
	Aver.	48.5	42.1	6.0	2.6	0.3
	Max.	61.8	36.7	16.0	4.7	1.0
5-6	Min.	52.6	21.2	6.5	0.7	0.3
	Aver.	56.5	29.9	10.0	2.5	0.6
	Max.	61.3	34.1	15.7	4.7	0.6
6-7	Min.	52.3	24.5	8.1	0.1	0.0
	Aver.	56.0	30.4	10.8	2.2	0.2
	Max.	72.0	39.1	15.2	3.5	0.2
7-8	Min.	45.2	21.1	6.7	0.0	0.0
	Aver.	54.4	32.5	11.6	0.1	0.06

In one instance in which blood counts were made by the physician before the patient came to the hospital, a low total count appeared, 7,000 two days before the paralysis and 3,000 on the day after paralysis. The differential count, however, showed a polymorphonuclear increase each time (81 per cent. and 58 per cent). Except for this one case which subsequently became one of our seventy-one, we have not seen a true leukopenia. One abortive case in a little girl 6 years of age showed counts of 5,600 to 11,800. In the youngest children the leukocyte counts have been highest, and the polymorphonuclear increase has been both relatively and actually more marked than in the older patients. A glance at the tables, however, will show that all ages have a high white cell count. Furthermore, the leukocytosis persists for a surprising length of time. In nine cases, for example, observed during the seventh week, the leukocytes numbered 15,000 to 19,500 with an average of 17,250.

The differential count presents a fairly constant picture. For comparison, a table of differential counts from normal individuals prepared

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by Schloss[11] is printed. There is obviously in poliomyelitis an increase in polymorphonuclear cells of 10 to 15 per cent. above the normal. This increase appears irrespective of the patient's age, though the youngest children have shown perhaps the highest polynuclear counts. Contrary to the observations of Müller and Gay and Lucas, we have found a diminution of lymphocytes. In general, they have been from 15 to 20 per cent. below the normal percentages. Notwithstanding, however, the great variation which these cells present in relation to age, the finding of low lymphocyte percentages has been constant in all our cases of poliomyelitis.

No abnormality was noted in the number of transitional and large mononuclear cells. In a good many cases there has been an increase in eosinophils. This finding, however, has not been sufficiently constant to justify further comment. No counts were made of the red cells, nor were any hemoglobin estimations recorded. There has been nothing, however, in the appearance of the patients to suggest an anemia resulting from the disease; nor did the stained erythrocytes look abnormal. No actual counts were made of blood platelets, but the impression was gained in the course of many differential counts that these elements were somewhat increased.

To sum up, then, we have found in the blood of patients with poliomyelitis a constant and marked leukocytosis. In several instances the count has been as high as 30,000. In only one case has there been a definite leukopenia. Besides the increase in total cells, there has been an equally constant increase of polymorphonuclears of 10 to 15 per cent. above the normal, and a diminution of lymphocytes of 15 to 20 per cent. The other forms of leukocytes have shown no abnormalities.

What the significance of this leukocytosis, and especially the polynucleosis, may be, is at present not clear. In view of the fact that the brunt of the body's attempt at defense falls on the lymphatic system, and that the cells which are marshalled at the point of attack seem to be lymphocytes, one might be led at first thought to expect a lymphocytosis in the blood. On the other hand, it is conceivable that the lymphocytes are relatively few in the circulation because they are constantly being withdrawn to meet the invasion of the virus in the spinal cord. It will require, however, a more fundamental knowledge of the kind of reaction the body develops to this type of infecting agent before a correct explanation of the blood picture can be made. At all events, while the blood picture in poliomyelitis is perhaps not any more specific than are the changes in the spinal fluid, it is at least helpful. If taken in connection with other available evidence, a leukocytosis of 15,000 to 30,000 is

11. Schloss: "The Normal Percentages of the Different Varieties of Leukocytes in Infants and Children," Arch. Int. Med., 1910, vi, 638.

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distinctly suggestive of the disease in question, especially if the polymorphonuclear cells are increased at the expense of the lymphocytes.

NOTE.—Since this paper has reached the proof, certain additions to our knowledge of the pathology of poliomyelitis have appeared which indicate definitely the general systemic nature of the infection. In view of the finding in many viscera of areas of focal necrosis with many polynuclear phagocytes, an explanation for the polymorphonuclear cytosis in the blood is at once suggested. Furthermore, the appearance of many disintegrating lymphocytes lends support to the idea that rapid destruction of lymphocytes underlies the lymphopenia. Both these explanations for the blood picture are offered purely as hypotheses.

Sixty-Sixth Street and Avenue A.

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STUDIES IN BONE METABOLISM: THE ETIOLOGY OF NON-PUERPERAL OSTEOMALACIA*

F. H. McCRUDDEN, M.D., AND H. FALES, S.B.
NEW YORK

The present investigation is a further contribution to the study of mineral metabolism and is designed to throw more light on the etiology and process in osteomalacia. A correct interpretation of this process is of the greatest importance in an understanding of the metabolism of bone since the two views regarding the nature of the osteoid tissue in osteomalacia — the one, that it is an old bone from which the mineral constituents have been dissolved out, and the other that it is new bone poor in mineral constituents — are based respectively on the two opposing views regarding the metabolism of bone — the one, that bone is dead tissue not undergoing metabolism, the other, that it does have a metabolism. Anatomical investigations not having been successful in deciding between these two views, chemical methods were applied, which led to the conclusion that bony tissue, like other tissue, is continuously being destroyed and replaced by new, and that osteomalacia is due to a disturbance of the balance whereby the new tissue is poor in calcium salts and therefore soft.[1] A study of the etiology led to the conclusion that the starting point is an increased catabolism of bony tissue due to a need of calcium salts elsewhere and that the tendency of physiological activity to overproduction which is responsible for the production of antitoxins and acquired immunity, is also an important factor in leading to the long-continued, increased catabolism of calcium after the original need

*From the Hospital of the Rockefeller Institute for Medical Research, New York.

1. In view of these findings it is interesting to note that v. Recklingshausen has recently been obliged to retract his interpretation of the *Gitterfiguren*, which was largely responsible in upholding the opposing theory, and to admit that it is not possible to differentiate morphologically between old bone from which the mineral salts have been removed and new bone poor in lime salts (von Recklinghausen: Untersuchungen über Rhachitis und Osteomalacie, 1910. See especially Chapter xiii and pages 343 and 346).

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has ceased to exist.[2] The present investigation is the result of an excellent opportunity of studying a rare case of non-puerperal osteomalacia and was designed to test the above theory of the nature of the process, and to investigate the relation of the need of calcium salts and the tendency of overproduction to the etiology.

The following is an extract of her history and physical examination:[3]

CASE REPORT

History.—The patient, a woman aged 42 and unmarried, dates her illness to 1891 when she noted a hard, painless nodule about the size of a bean on the right side of the lower jaw. Six months later the growth, which had by then increased to the size of a hen's egg, was removed together with a piece of the jaw. In 1895 she began to suffer from loss of appetite, vomiting, obstinate constipation and severe neuralgia in the face and upper jaw and began to walk with difficulty. She noticed that her ankles would "bend in and out" on walking "as if they were made of rubber." Then a gradual stiffening of the back and stiffness and creaking of the knees developed, so that, after five months, she could get about only with the greatest difficulty. In the fall of this year small, hard, painless nodules were noticed on her shin-bones. The largest, on the left, was about the size of a walnut. In 1897 she was forced to give up work entirely. A year later, while riding a low tricycle, she fell and fractured the right thigh. This fracture, which she describes as causing her surprisingly little pain, laid her up seven weeks after which, and until ten weeks ago, she was able to move about by leaning on a chair. While laid up she had an attack of renal colic, i. e., intense colicky pain, radiating downward from her right kidney region. Three weeks later she passed several small.

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2. McCrudden: Chemical Analysis of Bone from a Case of Human Adolescent Osteomalacia, Jour. Biol. Chem., 1910, vii, 199; The Composition of Bone in Osteomalacia, Am. Jour. Physiol., 1906, xvii, 32; A Study of the Metabolism in Osteomalacia, Am. Jour. Physiol., 1905, xiv, 211; The Effect of Castration on the Metabolism, Jour. Biol. Chem., 1910, vii, 185; The Effect of Castration on the Metabolism in Osteomalacia, Am. Jour. Physiol., 1906, xvii, 211; Studies of Bone Metabolism, Especially the Pathological Process, Etiology and Treatment of Osteomalacia, THE ARCHIVES INT. MED., 1910, v, 596.

The gynecologists are at present inclined to the hypothesis that osteomalacia is a disease of the adrenals. The original basis for this hypothesis was the experience of Bossi, an Italian physician, who, on Dec. 16, 1906, and on four successive days, administered epinephrin in a case of osteomalacia. At the end of that time the patient had much less pain and was therefore discharged as cured. *Within four weeks of the first treatment* the journal containing publication of the results appeared. (Bossi: Nebonniere und Osteomalakie, Centralbl. f. Gynäk., Jan. 19, 1907, No. 31, p. 69.) A number of other just such experiments have since been reported in the literature. In all cases "cure" was reported soon after treatment and meant simply relief from pain. The rapidity with which tenderness and pain disappear at times, even without treatment in osteomalacia, is well known and does not indicate a cure of the disease. The case reported by Kaessman (Ein Beitrag zur Adrenalin Behandlung des Osteomalakie nach Bossi, Centralbl. f. Gynäk., 1907, xxxi, 1376) may be typical of what happens to some of these patients. Kaessman administered epinephrin daily from Sept. 6 to Sept. 11, 1907, and, on September 14, sent the patient away better but with instructions to keep up the treatment. Nine days later, on September 23, the patient returned worse than ever. The suprarenal theory does not seem worthy of very serious consideration.

3. She was under the direct care of Dr. Henry K. Marks of this hospital.

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gray stones. These attacks of pain recurred, at intervals of six to eight months. Usually, about three weeks after each attack, she would pass a stone about the size of a pea. Since May, 1904, she has had no attacks. Between 1900 and 1905



Fig. 1.—*X*-ray plate showing bony tumor from the left shin of a patient suffering from osteomalacia.

her symptoms did not progress. Then the stiffness of her knees and back increased and she noticed a painless enlargement near the lower edge of the spine. In 1907

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she began to grow deaf in the right ear. A year later there was difficulty in breathing following the appearance of a bony growth in the right nostril. In the spring of 1909 it was noticed that her left eyeball protruded and she complained of dimness of vision and seeing double. There was no pain. In 1909 small nodules were noticed on her forehead. About this time she fractured the second metacarpal bone of the right hand by shaking hands with a friend. In August, 1910.



Fig. 2.—Rarefaction in the second phalange of the index- and third fingers and in the fifth metacarpal bone of patient with osteomalacia.

while washing her hands, she dislocated the terminal phalanx of the left index-finger. For the last ten weeks she has been bedridden on account of increasing stiffness and weakness. She feels no pain on lying still but only on movement or when handled.

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Physical Examination of the Osseous System.—The patient is a poorly nourished, emaciated, distorted, middle-aged woman. She lies comfortably in bed except when she attempts to move. A slight ridge can be felt extending along the suture lines of the skull. There is a hard nodule about the size of a pea on the temporal bone behind the right ear, an indefinite firm swelling behind the left ear, and three small firm nodules on the frontal bone. The nodules are not tender and not movable. There are two firm swellings on either side of the raphé between the hard and soft palate. There is a sharp, bony exostosis on the outer edge of the outer third of the right clavicle. There is a marked rosary. The right costal margin is somewhat depressed. At the left over the costochondral margin there is an elevation which is tender. There is moderate kyphosis of cervical and upper dorsal vertebræ and marked scoliosis. The sacrum is very prominent. The iliac wings are thickened and nodular. The absence of about an inch of the lower jaw gives the face an asymmetrical appearance. The legs are emaciated. The left leg is somewhat longer than the right, owing to bowing out of the right femur just above the middle (site of the old fracture, over which is a large callus). The surfaces of both shins are roughened. Just below the middle of the left tibia, and involving chiefly the inner surface, is a hard, oval, bone-like mass 11 cm. long, and 13 cm. wide. It is not tender or movable and the skin is not adherent. On the second metacarpal joints of both hands, there are hard swellings. The terminal phalanx of the left index-finger is movable laterally.

X-Ray Pictures.—Examples of the bony tumors and bone rarefactions are seen in Figures 1 and 2, respectively. Figure 1 shows a tumor from the left shin. In Figure 2 examples of rarefaction may be seen in the second phalanges of the index-finger and third finger and in the fifth metacarpal bone.

Briefly summarized, the condition is one of osteomalacia following the appearance of multiple bony tumors.

EXPERIMENTAL DATA

A complete metabolism experiment of ten days' duration, in which the intake and outgo of nitrogen, calcium, magnesium, sulphur and phosphorus was determined, was carried out. The diet consisted of the mixed diet of the hospital, only foods that were not homogeneous enough to sample properly being excluded. The diet for a week preceding the experiment was the same as that used in the experiment, so that there was no abrupt change. The details of feeding, collecting excreta, sampling and analyzing have been described in earlier papers.[2] The diet follows:

NOVEMBER 17.—*Breakfast:* White bread, 51.8 gm.; oatmeal, 104.4 gm.; scrambled eggs, 32.9 gm.; coffee, 40.0 c.c.; milk, 430.0 c.c.; water, 200.0 c.c. *Luncheon:* Potatoes, 51.2 gm.; rice, 98.5 gm.; ham, 23.1 gm.; custard, 112.4 gm.; milk, 205.0 c.c.; chicken broth, 160.0 c.c.; water, 200.0 c.c. At 3:30 p. m.: milk, 250.0 c.c. *Supper:* Hamburg steak, 34.2 gm.; sweet potatoes, 23.6 gm.; banana, 44 gm.; milk, 130.0 c.c.; water, 228.0 c.c.

NOVEMBER 18.—*Breakfast:* Graham bread and butter from stock; oatmeal, 96.4 gm.; lamb chop meat, 14.6 gm.; coffee, 50.0 c.c.; milk, 470.0 c.c.; water, 360.0 c.c.; water (injection*), 300.0 c.c. *Luncheon:* Bread and butter from stock; chicken meat, 23.3 gm.; spinach, 29.7 gm.; rice, 41.4 gm.; water, 250.0 c.c.; chicken

*It was occasionally necessary to give an enema to move the bowels. Tap water was used and the amount used added to the food to be analyzed.

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broth, 160.0 c.c.; milk, 150.0 c.c. *Supper:* Bread and butter from stock; meat. 26.5 gm.; junket, 114.6 gm.; chicken broth, 100.0 c.c.; milk, 150.0 c.c.; water, 450.0 c.c.

NOVEMBER 19.—*Breakfast:* Oatmeal, 85.0 gm.; sausage, 33.0 gm.; water, 200.0 c.c.; coffee, 60.0 c.c.; milk, 100.0 c.c. *Luncheon:* Chicken, 25.9 gm.; rice, 79.2 gm.; custard, 48.1 gm.; Graham bread and butter from stock; water, 210.0 c.c.: milk, 210.0 c.c.; soup, 170.0 c.c.; gravy, 50.0 c.c. *Middle Afternoon:* milk, 250.0 c.c.; water, 390.0 c.c. *Supper:* Meat, 23.9 gm.; banana, 40.3 gm.; water, 150.0 c.c.; milk, 200.0 c.c.; soup, 160.0 c.c.

NOVEMBER 20.—*Breakfast:* Oatmeal, 93.2 gm.; eggs, 40.6 gm.; brown bread and butter from stock; water, 200.0 c.c.; coffee, 140.0 c.c.; milk, 355.0 c.c.; water (injection), 240.0 c.c. *Luncheon:* Brown bread and butter from stock; chicken, 46.9 gm.; rice, 63.8 gm.; spinach, 46.3 gm.; ice-cream, 41.0 gm.; soup, 135 c.c.; water, 200.0 c.c.; milk, 250.0 c.c. *Supper:* Brown bread and butter from stock; soup, 170.0 c.c.; milk, 500.0 c.c.; water, 200.0 c.c.

NOVEMBER 21.—*Breakfast:* Brown bread and butter from stock; oatmeal, 97.1 gm.; water, 200.0 c.c.; milk, 250.0 c.c.; coffee, 70.0 c.c. *Luncheon:* Beefsteak, 25.7 gm.; rice, 71.3 gm.; apple sauce, 112.3 gm.; bread and butter from stock; water, 200.0 c.c.; milk, 400.0 c.c.; soup, 170.0 c.c. *Supper:* milk, 185.0 c.c.; oyster soup, 170.0 c.c.; water, 200.0 c.c.

NOVEMBER 22.—*Breakfast:* Banana, 37.4 gm.; oatmeal, 88.5 gm.; water, 180.0 c.c.; milk, 350.0 c.c.; coffee, 70.0 c.c.; water (injection), 315.0 c.c. *Luncheon:* Chicken, 33.0 gm.; rice, 76.0 gm.; custard, 93.8 gm.; bread and butter from stock; water, 180.0 c.c.; soup, 180.0 c.c.; milk, 250.0 c.c. *Supper:* Meat, 21.8 gm.; sweet potato, 51.7 gm.; water, 200.0 c.c.; milk, 470 c.c.; broth, 150 c.c.

NOVEMBER 23.—*Breakfast:* Bread and butter from stock; oatmeal, 91.1 gm.; egg-milk-sherry mixture, 215.0 c.c.; water, 120.0 c.c.; coffee, 70.0 c.c.; milk, 350.0 c.c. *Luncheon:* Bread and butter from stock; rice, 91.1 gm.; custard, 93.1 gm.; chicken, 32.2 gm.; water, 200.0 c.c.; soup, 170.0 c.c.; milk, 250.0 c.c.; gravy, 25.0 c.c. *Supper:* Water, 200.0 c.c.; egg-milk-sherry, 235.0 c.c.; soup, 170.0 c.c.

NOVEMBER 24.— *Breakfast:* Bread and butter from stock; banana, 42.7 gm.; water, 220.0 c.c.; sausage, 49.4 gm.; coffee, 70.0 c.c.; milk, 100.0 c.c. *Luncheon:* Rice, 88.0 gm.; peas, 52.3 gm.; turkey, 41.5 gm.; milk, 225. 0c.c.; water, 210.0 c.c.; broth, 180.0 c.c. *During Afternoon:* milk, 250.0 c.c. *Supper:* Chicken, 24.3 gm.; apple sauce, 76.7 gm.; milk, 470.0 c.c.; broth, 80.0 c.c.; water, 210.0 c.c.

NOVEMBER 25.—*Breakfast:* Banana, 42.8 gm.; oatmeal, 94.6 gm.; water, 210.0 c.c.; milk, 510.0 c.c.; coffee, 70.0 c.c. *Luncheon:* Rice, 47.8 gm.; spinach, 43.8 gm.; chicken, 33.1 gm.; water, 210.0 c.c.; broth, 170.0 c.c.; milk, 250.0 c.c.; gravy, 55.0 c.c. *During morning:* Milk, 250. c.c. *Supper:* Bread, 16.4 gm.; lamb, 23.8 gm.; peaches, 64.0 gm.; egg-milk-sherry mixture, 230.0 c.c.; milk, 470.0 c.c.; water, 230.0 c.c.; broth, 160.0 c.c.

NOVEMBER 26.—*Breakfast:* Bread, 39.7 gm.; oatmeal, 67.0 gm.; beefsteak. 28.5 gm.; milk, 420.0 c.c.; water, 100.0 c.c.; coffee, 70.0 c.c. *Luncheon:* White sauce, 40.0 gm.; apple sauce, 68.2 gm.; bread, 25.6 gm.; water, 200.0 c.c.; milk, 225.0 c.c.; soup, 160.0 c.c. *During day:* Water, 200.0 c.c.

For the whole experiment: Graham bread, 536.6 gm.; white bread, 213.4 gm.; butter, 612.5 gm.; salt, 5.9 gm.

We neglected to weigh the patient at the end of the experiment, but she appeared to be steadily gaining in weight and strength and there was a considerable retention of nitrogen.

The accompanying tables give the results.

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TABLE 1.—METHODS OF ANALYSIS AND AMOUNTS USED

	Amounts Used for Analysis			Method of Analysis
	Urine c.c.	Food* gm.	Feces† gm.	
N	5	1	1	Kjeldahl
CaO	200	10	1	McCrudden[4]
MgO	200	10	1	McCrudden[4]
S	50	2	2	Folin[5]
P_2O_5	50	2	1	Neumann[6]

* Total weight 3104.7 gm. † Total weight 246.7 gm.

TABLE 2.—ANALYSIS OF URINE DAY BY DAY *

Day	N gm.	CaO gm.	MgO gm.	S gm.	P_2O_5 gm.
1	8.08	0.291	0.106	0.590	1.692
2	8.40	0.293	0.119	0.648	1.680
3	10.28	0.333	0.139	0.734	1.840
4	10.08	0.379	0.107	0.716	2.020
5	9.56	0.315	0.123	0.656	1.948
6	10.56	0.350	0.131	0.730	1.880
7	10.92	0.330	0.129	0.804	2.072
8	11.52	0.298	0.129	0.762	1.996
9	10.76	0.477	0.045	0.788	2.048
10	9.52	0.278	0.123	0.684	1.936
Total..	99.68	3.344	1.151	7.112	19.112

* The urine analyses were made in duplicate.

TABLE 3.—COMPARISON OF ANALYSES OF URINE, FOOD AND FECES *

	N gm.	CaO gm.	MgO gm.	S gm.	P_2O_5 gm.
Urine total	99.68	3.344	1.151	7.112	19.112
Feces total	11.72	18.80	3.40	1.024	15.57
Total outgo	111.4	22.14	4.55	8.14	34.68
Total intake (food)	121.4	21.17	4.69	10.03	35.55
Retention	10.0	....	0.14	1.89	0.87
Loss	....	0.97	....	....	....
Per cent. retained.	+8.3%	—4.6%	+3.0%	+18.8%	+2.4%

* The food and feces analyses were made in triplicate.

DISCUSSION

For a correct interpretation of the figures, the percentage accuracy of the results should be known. It is not necessary to give the weights of all the final precipitates with a discussion of the accuracy of each since the approximate accuracy of the methods can be judged by anyone familiar with them. The last figures in each case in the reported balances are probably correct to within a few figures — that is, the retention of nitrogen lies between 9.98 and 10.02 gm.; the loss of calcium oxid between 0.93 and 1.01 gm., etc.[7]

4. McCrudden, F.: The Quantitative Separation of Calcium and Magnesium, etc., Jour. Biol. Chem., 1910, vii, 83.

5. Folin, O.: Sulphate and Sulphur Determinations, Jour. Biol. Chem., 1905, i, 131.

6. Neumann, A.: Einfache verraschungs Methode, etc., Ztschr. f. physiol. Chem., 1902, xxxvii, 131; Nachträge zur Säuregemisch-Verraschung, Ztschr. f. physiol. Chem., 1904, xliii, 35.

7. The probable limits of experimental error are about as follows:

	N.	S.	CaO	MgO	P_2O_5
Total urine	±0.2	±0.03	±0.01	±0.01	+0.05
Feces	±0.02	±0.01	±0.03	±0.02	±0.07

Taking the larger of these two sets as the possible error for the total outgo, we get:

Total outgo	±0.2	±0.03	±0.03	±0.02	±0.07

For food the figures are about as follows:

Food	±0.2	±0.05	±0.04	±0.02	±0.07

Taking the larger of each of these two sets for the error in the balance, we get

Balance	±0.2	±0.05	±0.04	±0.02	±0.07

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It is plain that there was a definite loss of calcium accompanied by a retention of all other elements and that the amounts lost and retained were outside the limits of experimental error. The question arises: Is the amount lost a significant amount? The significance of the loss depends partly on the amount metabolized, partly on the relative loss of other substances. The amount of calcium oxid lost in twelve days was about 1.0 gm., nearly 5 per cent. of the amount in the food, and over three times the average daily amount in the urine, and is probably lost chiefly through the urine for the amount in the urine is nearly double the normal.[8] Furthermore, everything else was being retained, the retention running from 3 per cent. in the case of magnesium to 19 per cent. in the case of sulphur. These relations cannot be normal. Towle[9] has pointed out that there is a parallelism in the metabolism of nitrogen and calcium in health and in disease unless very large quantities of calcium are especially given. And this has been found to be true for other elements. The balance given in Table 4 was found in the case of a healthy boy.

TABLE 4.—METABOLISM OF A HEALTHY BOY

	N.	S.	CaO	MgO
Total intake	86.10	6.138	14.99	3.26
Total outgo	71.46	5.222	12.42	2.53
Balance	+14.64	+ .916	+ 2.57	+ 0.73
Per cent.	+17.0	+14.9	+17.1	+22.4

The results of Hoffström,[10] who studied the metabolism of a pregnant woman for a long period, showed similar parallelism in retention between nitrogen and mineral salts.

The retention of sulphur and magnesium is greater in proportion than that of nitrogen and corresponds to a growth of tissue richer in sulphur and magnesium than any of the soft tissues — such a tissue as bone. This condition is in accord with McCrudden's conception regarding the process going on, namely, that bony tissue is being catabolized and that the new bone laid down to replace it is poorer in calcium

8. The loss of calcium and retention of magnesium and sulphur are not so great in this experiment as in the experiment on which my conception of the etiology and process was based. In one of those experiments there was an average daily loss of nearly 1 gm. of CaO (Am. Jour. Physiol., 1906, xvii, 211), and Berger (Presse méd., 1905, xiii, 249), speaks of a case in which he analyzed the urine only and found 9.0 gm. CaO per day. The patient was in relatively good condition at the time of the present investigation, probably as a result of the good hygienic environment. If we could have studied her condition when the process was more active, greater losses might have been expected.

9. Towle, C.: Calcium Metabolism with Special Reference to Exophthalmic Goiter, Am. Jour. Med. Sc., 1910, cxl, 100.

10. Hoffström, K.: Eine Stoffwechseluntersuchung während der Schwangerschaft, Skandin. Arch. f. Physiol., 1910, xxiii, 326.

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but richer in sulphur and magnesium than normal bone, similar to osteoid tissue plus magnesium salts.

The clinical course of this case of osteomalacia is in accord with McCrudden's conception of the etiology. The various bony tumors need lime salts for their growth and, as already pointed out in earlier papers, the bones act as a reservoir of lime salts from which the body may draw in time of need.[11]

The tendency of the body to overproduction, to the continuation of an activity once started after the need for activity has ceased to exist, is shown in this case. The hard tumors which were probably responsible for starting the increased catabolism of bone were no longer actively and rapidly growing, yet the flux of calcium salts from the bones continued and, as shown by the metabolism experiment, the calcium was simply excreted. There are many analogies for this process. Under the name of "functional inertia," Harris[12] points out that it is as fundamental and important an attribute of living matter as irritability itself. He defines functional inertia as "that property of protoplasm whereby living matter contrives to remain in a functional *status quo ante* notwithstanding that it has received a stimulus, or, having responded to the stimulus, it contrives to exhibit its functional activity for a certain time after the stimulus as a form of energy has ceased." The condition may be compared with that of a heavy door which, when at rest, requires a certain time to get into full swing after it has been pushed and, when moving, takes time to stop if held. Of examples of latent period in starting activity, there is the latent period of muscular contraction, that occurring after glands are stimulated through their nerves and before they begin to secrete, and that occurring before the heart-beat begins to accelerate its rate when the augmentor or accelerator nerves are stimulated. Of examples of continued activity after the original stimulus has ceased

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11. Further evidence to this effect has recently appeared: King, Bigelow, and Pearce (Experimental Obstructive Jaundice, Jour. Exper. Med., 1911, xiv, 148) have shown that the calcium content of the bones decreases in cases of obstructive jaundice in which calcium is needed to neutralize the toxic effects of the bile acids. Schmidt (Verhandl. d. deutsch. path. Gesellsch., 1909, xiii, 3) has pointed out that the decrease of calcium in the bony system in rickets is accompanied by an increase of this element in the soft tissues such as muscle and liver, and Higbee and Ellis, in a recent paper on osteitis deformans (Jour. med. Research, 1911, xxiv, 43)—a condition which is recognized as one of localized osteomalacia—state that calcification of the arteries is so common in these cases that it has been mentioned as a possible etiological factor.

Dr. Charles F. Painter of Boston has called McCrudden's attention to the occurrence of a late variety of non-rachitic bow-legs occurring in children about the time the epiphyses begin to harden. Is it not possible that a flux of calcium might be started by the demand for this element by the hardening epiphyses analogous, in a way, to the physiologic osteomalacia noted by Hanau and Wild in the pelvic bones of pregnant women?

12. Harris, D.: The Functional Inertia of Living Matter, London, 1908.

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may be mentioned the continuation of accelerated heart action for a time after stimulation of the augmentor nervous apparatus has ceased, the continued overproduction of free receptors which, according to Ehrlich's theory, is the explanation of acquired immunity and the overproduction which Weigert has pointed out is characteristic of all tissue repair. These examples are analogous to the continued catabolism of the lime salts of bone after the original stimulus for this process has ceased.[13] It is not improbable that overproduction may be another example of the factors of safety with which, as Meltzer[14] has pointed out, the body is so well endowed.

The recurrence of attacks of osteomalacia earlier and earlier in succeeding pregnancies, and the increasing severity of subsequent attacks, make one think of the more severe and rapid production of symptoms of certain diseases: e. g., serum disease and others after a previous inoculation — a kind of allergy,[15] or, to put it still more broadly, "a cell stimulated to perform a certain act not only continues to perform that act for some little time after the stimulus has ceased, but, what is more, on a second occasion a slighter stimulus will induce the like series.[16]

It ought to be emphasized that the conception regarding the etiology and process in osteomalacia outlined here is, of course, based *not on these theories* of functional inertia and allergy, *but on the facts* observed in chemical, clinical and anatomical investigations. The analogies are cited merely to show that, in all probability, the process in osteomalacia is but a special example of certain more general laws governing the *modus operandi* of living matter.

SUMMARY

The findings in the present case of osteomalacia are, then, in accord with the views outlined in this and previous papers[2] regarding the process and etiology of osteomalacia and give further support to these views. The loss of calcium and retention of magnesium and sulphur are in accord with the conception of the process in osteomalacia, as one in which the bone is undergoing metabolism but in which the new bone laid down is an osteoid tissue poor in lime salts but richer than normally in magnesium. The presence of bony tumors preceding the osteomalacia is in accord with the conception that the starting point in osteomalacia can

13. It is not improbable that another example of such overproduction or functional inertia is the change in the metabolism brought about by morphinism which enables the body to destroy increasing amounts of morphin but which continues and has a damaging effect on the organism itself if the drug is suddenly withdrawn.

14. Meltzer, S. J.: The Factors of Safety in the Animal Structure and Economy, Harvey Society Lectures, 1906-1907.

15. v. Pirquet, C.: Allergy, THE ARCHIVES INT. MED., 1911, vii, 259 and 382.

16. Adami: The Principle of Pathology, 1910, i, 195.

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usually, if not always, be traced to a demand for calcium salts. And the continued excretion of an excess of calcium when the bones really need this element is in accord with the conception regarding the importance of overproduction in bringing about the softening of the bone.

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THE INFLUENCE OF KIDNEY EXTRACTS ON THE BLOOD-PRESSURE

SUPPLEMENTARY NOTE *

RICHARD M. PEARCE, M.D.
NEW YORK

The study of extracts of the kidney is of importance in connection with that phase of the theory of the internal secretion of the kidney which ascribes to such a hypothetical secretion a part in the pathology of those lesions of the cardiovascular system characterized by hypertension. In an earlier investigation,[1] I have presented in detail observations indicating that the dog's kidney, unlike that of the rabbit and some other animals, does not contain a pressor substance, and demonstrating conclusively that it does contain a powerful depressor substance which is present also in the urine. In view of the fact that the pressor substance of other organs, as for example the adrenal, is constant for all mammals, these observations failed to support the universal application of that phase of the theory of internal secretion which ascribes to the kidney a pressor function.

The later observations of Bingel[2] and his associates rendered necessary, however, on account of the method which they employed, a reinvestigation of this question.

These investigators found that a pressor substance could be demonstrated constantly in the precipitate obtained by saturating the autolyzed kidney extract with ammonium sulphate. They worked chiefly with the kidney of the pig, but studied also the kidneys of various other animals without, however, special attention to the dog's kidney. Their method was as follows:

The cortex of pigs' kidneys obtained in a fresh condition from a slaughter-house was washed with salt solution, hashed, ground with quartz sand in a mortar and after the addition of infusorial earth, the juice was expressed under a pressure of 450 atmospheres. It was then allowed to autolyze under xylol for from six to fourteen days at a

*From the Department of Research Medicine, University of Pennsylvania; aided by a grant from the Rockefeller Institute for Medical Research.

1. Pearce, R. M.: An Experimental Study of the Influence of Kidney Extracts and of the Serum of Animals with Renal Lesion on the Blood-Pressure, Jour. Exper. Med., 1909, xl, 430.

2. Bingel, A., and Strauss, E.: Ueber die blutdrucksteigernde Substanz der Niere, Deutsch. Arch. f. klin. Med., 1909, xcvi, 476; Bingel, A., and Claus, R.: Weitere Untersuchungen über die blutdrucksteigernde Substanz der Niere, ibid., 1910, c, 412.

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temperature of 37 C. and, after removal of the suspended particles, was half saturated with ammonium sulphate. The fluid obtained after filtration was then completely saturated and the precipitate after dialysis was used for injection into the rabbit.

This method applied to the dog's kidney has failed, in my hands, to yield an extract which, when injected into the dog, is capable of producing a pressor effect. Extracts of the whole kidney, as of the cortex only, produced no change in the blood-pressure. Likewise, if the stage of autolysis was omitted, the extract obtained by complete saturation with the ammonium salt was inert. On the contrary, the juice obtained by Buchner press and not autolyzed or treated with ammonium sulphate gave a depressor effect. It is evident, therefore, that the method of Bingel, while it removes the depressor substance, fails to demonstrate the presence of a pressor substance. This conclusion is not, however, to be regarded as a criticism of Bingel's method, but only as a demonstration of the fact that a pressor substance, if it exists in the dog's kidney, cannot be isolated by the method of extraction which he describes.

It has been possible, however, to approach this problem in another way. As I have shown elsewhere,[3] the depressor substance normally present in dog's urine frequently disappears from the urine in the severe forms of nephritis caused by uranium nitrate and potassium chromate. It was assumed, therefore, that if this substance disappeared from the urine it would likewise be absent from the kidney, and if the kidney contained a pressor substance normally masked by the depressor substance, the pressor substance would under such conditions be demonstrable in the kidney extract. Experimental nephritis has therefore been produced by the use of uranium nitrate and potassium chromate, and the urine of the animals, obtained after three to six days, the usual period of disappearance, has been injected daily into normal dogs. When the depressor substance of the urine was found to be absent, the animal was killed, its kidney washed free of blood with normal salt solution, and the juice expressed with the aid of a Buchner press.

In several very carefully planned experiments, representing uranium and chromium nephritis, it was possible to obtain extracts of the kidney at the time of total disappearance of the depressor substance from the urine, but only two extracts were of value in the study of the point in question; one of these was entirely devoid of depressor action and the other only slightly depressing. Neither, however, gave the slightest evidence of the existence of a pressor substance. These experiments, though few in number, would therefore appear to indicate that the non-

3. Pearce, R. M.: The Depressor Substance of Dog's Urine and Its Disappearance in Certain Forms of Experimental Nephritis. Jour. Exper. Med., 1910, xii. 128.

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demonstration of a pressor substance in the dog's kidney is not to be explained as a masking of this substance by the more powerful depressor substance. The pressor substance, apparently, does not exist in the kidney of this animal.

SUMMARY

The method used by Bingel and his associates to demonstrate a pressor substance in kidney extracts fails to reveal such a substance in extracts of the dog's kidney. Extracts of the kidneys of dogs with experimental uranium and chromium nephritis, prepared at a time when the urine is free of the normally present depressor substance, have no pressor effect.

These observations would appear to demonstrate conclusively that the dog's kidney does not contain a pressor substance which is masked by the more powerful depressor substance. They also fail to support that phase of the theory of internal secretion which assumes that the kidney furnishes a pressor substance of importance in the pathology of cardiovascular disease.

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AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE DEARBORN AVENUE
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March, 1912

HEREDITY IN HETEROGENEOUS HYBRIDS[1]

JACQUES LOEB

The Rockefeller Institute, New York

NINETEEN FIGURES

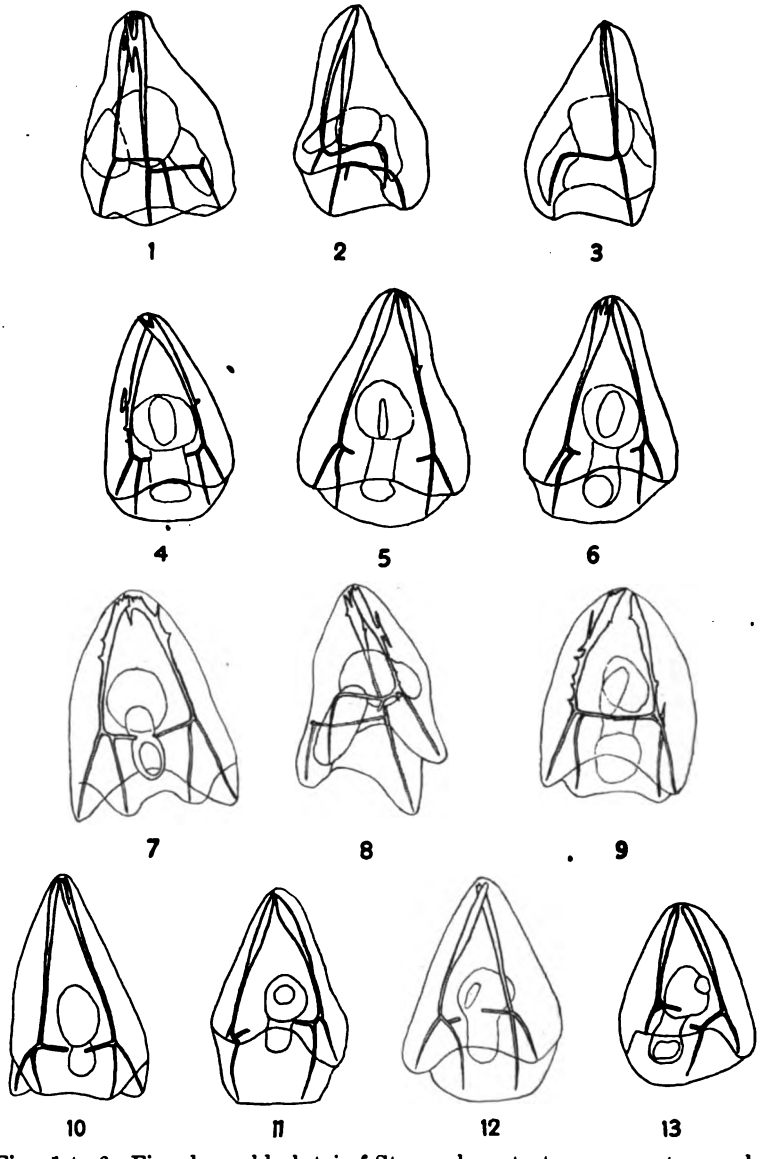
1. The study of heredity in embryos offers in one respect a wider field than that in adults inasmuch as heterogeneous hybrids rarely reach the adult stage. Eight years ago I found a method by which the eggs of the sea-urchin can be fertilized by the sperm of starfish, ophiurians and holothurians. The larvae are purely maternal, namely plutei. The results were confirmed by Godlewski for the fertilization of the egg of the sea-urchin by the sperm of the crinoid. It is well known that if we cross two homogeneous forms, e.g., two forms of sea-urchins, the paternal influence can be clearly seen in the pluteus stage. Since I have never published the figures of my experiments on heterogeneous hybridization, I may supplement my former statements with a few drawings. Figs. 1 to 6 are camera drawings of plutei of Strongylocentrotus purpuratus produced by artificial parthenogenesis. The plutei are, of course, in every detail identical with the plutei obtained if these eggs be fertilized with sperm of their own species. Figs. 7 to 9 are drawings of five days old plutei of Strongylocentrotus purpuratus ♀ and Strongylocentrotus franciscanus ♂. They differ from the pure breeds of S. purpuratus in several characters of the skeleton which exist in the pluteus of franciscanus but are absent from purpuratus, namely the greater roughness of the skeleton, the presence of cross bars and the greater length of the arms.[2] In figs. 10 to 13 are shown the five days old plutei of the egg of S. purpuratus fertilized with the sperm of the starfish (Asterias). It is obvious that the latter

[1] Prepared for the Whitman Memorial Volume but received too late to be included.

[2] Loeb, King and Moore, Arch. f. Entwicklungsmechanik, vol. 29, 1910.

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Figs. 1 to 6 Five days old plutei of Strongylocentrotus purpuratus produced by artificial parthenogenesis.

Figs. 7 to 9. Five days old plutei of Strongylocentrotus purpuratus ♀ and Strongylocentrotus franciscanus ♂.

Fig. 10 to 13 Five days old plutei from Strongylocentrotus purpuratus ♀ and Asterias ♂.

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plutei are purely maternal. It should, however, be borne in mind, that the objection might be raised that the presence of the skeleton in the sea-urchin pluteus might be dominant over its absence in the starfish larva. I have thus far vainly tried to produce a starfish larva with a pluteus skeleton by the hybridization of the two species.

We are therefore compelled to state that the hybrids between the sea-urchin egg and the starfish sperm represent more closely the purely maternal form than do the hybrids between two sea-urchins, which always show paternal characters.

2. It is well known that Herbst and Tennent have made experiments in which the paternal influence in the hybrid embryo was diminished. Tennent states that in the cross between Hipponoe and Toxopneustes, Hipponoe characters become dominant in sea water of a high OH concentration and Toxopneustes characters in sea water of a low OH concentration.[3] The amount of acid or alkali Tennent needed to accomplish his result was very small; namely about 2 cc. $\frac{N}{10}$ acetic or hydrochloric acid to 500 cc. of sea water. A. R. Moore, W. R. King and myself made a number of experiments in which the hybrids between S. purpuratus and S. franciscanus were raised in sea water to which varying quantities of HCl or acetic acid or NaHO were added (from 0 to 0.4 cc. $\frac{N}{10}$ acid or NaHO to 50 cc. of sea water). We were able to retard or accelerate the rate of development, but the character of the hybrid remained absolutely unaltered.

I wish to call attention to the necessity of sterilizing the pipettes by boiling them after each experiment, instead of sterilizing them by rinsing in distilled or fresh water as is often done.

3. Moenkhaus measured the rate of segmentation in hybrid fish eggs and found that the rate for the first five cleavages is determined by the egg.[4] The egg of Ctenolabrus segments about forty minutes after impregnation with sperm of its own kind, while the egg of Batrachus tau, if fertilized with the sperm of the same species, segments after about eight hours. If the egg of Batrachus be fertilized with the sperm of Ctenolabrus it also does not

[3] Tennent, Publication 132, Carnegie Institution, 1910.

[4] Moenkhaus, Am. Jour. of Anat., vol. 3, p. 29, 1904.

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segment until after eight hours.[5] I have repeated these experiments in a number of fish hybrids and confirmed Moenkhaus' results. The same facts are observed in the rate of development of hybrid embryos of echinoderms. What is the meaning of this fact? I believe that it finds its explanation through artificial parthenogenesis. These latter experiments have shown that the spermatozoon does not cause the development by carrying an enzyme or katalyzer into the egg, which the latter needs in order to develop, but causes the development by altering the surface layer of the egg. If the development of the egg were caused by an enzyme carried into the egg by a spermatozoon, the rate of cleavage of slowly developing eggs should be accelerated by a spermatozoon of a species developing at a faster rate. The egg however behaves exactly as we should expect from the fact that the spermatozoon removes only certain obstacles for the development of the egg but does not cause its development by carrying an activating enzyme. In order to cause the parthenogenetic development of the sea-urchin egg two different agencies are required and I have been able to show that the spermatozoon also causes the development of the sea-urchin egg by two agencies which act analogously to the agencies employed in my method of artificial parthenogenesis.[6] F. Lillie has found the same in Nereis.[7] It was therefore to be expected that the rate of development of embryos was determined by the egg. This view meets with a difficulty in the fact that, with a few exceptions, the later development of hybrids is as a rule retarded. But this difficulty is only an apparent one since the retardation is due to an entirely secondary condition: namely that most hybrids are sickly and not able to hatch or reach an adult state.

This is most strikingly the case in the heterogeneous hybrid, e.g., in the cross between the sea-urchin and the starfish. As I pointed out long ago the larvae die mostly in the gastrula stage,

[5] The acceleration of segmentation which Newman observed in the egg of Fundulus majalis fertilized by the sperm of Fundulus heteroclitus is too small to influence our conclusions.

[6] Loeb, Die chemische Entwicklungserregung des thierisches Eies. Berlin, 1909, and Das Wesen der formativen Reizung, Berlin, 1909.

[7] F. R. Lillie, Jour. of Morph., vol. 22, 1911.

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and possibly one egg in a million reaches the pluteus stage. The development of the pluteus is in such cases always retarded.

We find such a retardation not only in the case of heterogeneous hybridization but occasionally also in the case of crosses between closely related forms. While the hybrid purpuratus ♀, franciscanus ♂ is vigorous, the hybrid franciscanus ♀, purpuratus ♂, is sickly and reaches the pluteus stage only rarely and slowly.

The rate of development of the embryo is a function of the velocity of certain chemical processes which are linked together like the wheels in a mechanical machine. If one of such processes be retarded the rate of development of the whole embryo is likely to be retarded; and if the linkage of the various chemical processes become disturbed the embryo is likely to be sickly. The further apart the species are from which the two sex cells originate the greater the likelihood that the rate of development is retarded and that the hybrid embryo is sickly. Why is the development not retarded from the beginning? Possibly for the reason that it requires some time before the spermatozoon can cause the formation of a sufficient amount of harmful substances to cause a retardation of the development of the egg.

4. Moenkhaus found that the eggs of bony fishes can be easily impregnated with foreign sperm but that they do not develop very far. Thus he states, that the hybrids between Menidia and Fundulus heteroclitus "never go beyond the closure of the blastopore." I have been able to raise the hybrid between Fundulus heteroclitus ♀ and Menidia, Ctenolabrus and Stenotomus ♂ in large numbers beyond this stage. These hybrids lived a month or longer, formed hearts, blood vessels, eyes, and fins but never hatched. With a few exceptions no circulation was ever established although the heart beat for weeks.

Figs. 14 to 16 show three different hybrids of Fundulus heteroclitus ♀ and Menidia ♂, from a lot fertilized the 12th of June. The camera drawings were made from the living material the 2d, 3d and 12th of July. At that time the pure breed of Fundulus heteroclitus fertilized on the same date were already hatching. The hybrid embryos had formed the pigment characteristic for the pure breed of Fundulus heteroclitus. But the anomalies of

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the embryos are very obvious. The embryos are rather small, owing to the slowness with which they digest the yolk. Their eyes are abnormal and approach the cyclopean condition. In many specimens only irregular masses of pigment indicate where the eyes should be. The head is comparatively small and not bent as is characteristic for the pure breed. The heart is developed but corresponds to an early stage in the development. It beats regularly and at an almost normal rate. The main blood vessels exist and haemoglobin is formed but the creeping of the pigment cells upon the blood vessels does not take place.

Years ago I found that the marking of the yolk sac of Fundulus and of the embryo is caused by the creeping of the chromatophores upon the blood vessels. I showed that this phenomenon is due to a tropism which depends upon the circulation. When the circulation was suppressed pigment was developed but the chromatophores did not creep upon the blood vessels. At that time I had succeeded in suppressing the circulation for a few days.[8] In the new experiments the hybrid embryos lived for a month or more with pigment but without a circulation. They demonstrate the correctness of my former statement, inasmuch as the creeping of the chromatophores upon the blood vessels did not take place. They also confirm the statement, that the formation of pigment cells is independent of the circulation. Newman seems to hold the opposite view, but he evidently did not test his assertion experimentally.

These hybrids were also smaller than the pure breeds of the same age, owing to the fact that the yolk is less rapidly digested in the hybrids than in the pure breeds. This is a very important link in our conclusions on heredity. The development of hereditary characters is the result of the nature and the velocity of chemical reactions between the mass of yolk on the one hand and the substances in the nucleus, especially the chromosomes, on the other. If two closely related forms be crossed, the chemical reactions are not materially different in quality and velocity from those of the pure breeds. But when distant forms are crossed it is to be expected that greater differences in the nature and the

[8] Loeb, Jour. of Morph., vol. 8, 1893; Woods Hole Lectures.

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rate of chemical reactions will be found and the outcome will be pathological embryos and very likely a suppression of the paternal influence. The disturbance is the same in practically all the heterogeneous hybrids. I have also produced the crosses between Ctenolabrus ♂ and Fundulus heteroclitus ♀ and between Stenotomus ♂ and Fundulus heteroclitus ♀. In all cases the result was about similar to the one described here. In all cases there was a consumption of yolk, development of an embryo, of pigment, of a heart beat, of eyes, lenses, ears, fins; but, with rare exceptions about which we are to speak later, there was no circulation. The number of relatively good embryos was very large in the cross between Fundulus heteroclitus ♀ and Menidia ♂ (where about 90 per cent formed embryos that lived for about a month); it was much smaller in the cross between Fundulus heteroclitus ♀ with Ctenolabrus ♂. One word should be said in regard to the development of the head in these embryos. In later stages it is often abnormally small in comparison with the body. The reason for this is that, although at first the head of these heterogeneous hybrids develops normally, sooner or later its development stops and often phenomena of degeneration set in, especially in the eyes. The body of such larvae however continues to grow.

5. From what was said before, I reached the conclusion, that these hybrid larvae between Fundulus ♀ and Menidia ♂ were in reality pure breeds, namely Fundulus heteroclitus larvae whose development was retarded through some interference with the normal chemical reactions in the egg; and that the abnormalities described were in no way hybrid characters. It occurred to me that it might be possible to produce similar larvae from pure breeds of heteroclitus eggs, if the latter were compelled to develop under an abnormal chemical condition. For this purpose the following experiment was made. Eggs of Fundulus heteroclitus were put immediately after fertilization into closed Erlenmayer flasks, each of which contained 50 cc. of sea water to which various amounts of a 0.01 per cent solution of NaCN were added, from 0 to 10 cc. The eggs remained in these solutions about a month. In the mixture of 2 cc. 0.01 per cent NaCN and 50 cc. of sea water, embryos were found which in every way resembled the hybrids

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between Menidia ♂ and Fundulus heteroclitus ♀. (See figs. 17 and 18.) Their eyes were poorly developed, they had a tendency to form cyclopean eyes, the yolk was incompletely digested and the embryo too small. The heart was formed and beat, but no circulation was established. Pigment was formed (in the drawing most of the black pigment cells on the yolk sac were not indicated, since the drawing was intended for another purpose). The head is not bent against the body, and so on. In all respects these sickly embryos of pure breed resemble the hybrids between Fundulus heteroclitus ♀ and Menidia ♂.

Since NaCN acts through a retardation in the rate of oxidation, the idea might be expressed, that in heterogeneous hybrids oxidations are interfered with. It is not safe to accept such an idea until it has been tested experimentally.

6. The idea that the heterogeneous hybrids in fish are purely or at least essentially maternal finds support in the fact that a small number of these hybrids develop more normally than those thus far mentioned. In a small number of such hybrids circulation is established, though as a rule rather late and often for a few days only. But these embryos develop rather normally and are, as far as any present observations go, purely maternal. I have had many such hybrids but I will give only one camera drawing (fig. 19), representing a twenty-five day old hybrid between a male scup and a female Fundulus heteroclitus. The yolk is in this case pretty well digested.

7. While it is the rule that in the case of heterogeneous hybridization heredity is purely maternal, it is possibly not without exception. I have however thus far found only one paternal character which is possibly transmitted to a heterogeneous hybrid. The yolk sac of the egg of Fundulus heteroclitus forms branched red chromatophores which are not found on the yolk sac of Menidia. In two eggs of Menidia fertilized by sperm of Fundulus heteroclitus a few red chromatophores were observed. It is difficult to get this cross and I give this observation with some hesitancy.

8. Kupelwieser and Baltzer account for the fact that heterogeneous hybrids are purely maternal by the assumption that the

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chromosomes of the sperm are thrown out of the egg or disintegrate. This is not in harmony with the observations of Moenkhaus for the hybrids between Menidia ♂ and Fundulus heteroclitus ♀, and with those of Godlewski for the hybrids between sea-urchin and crinoid. I am not in a position to decide the differences in the observations of these authors. The observations mentioned in the preceding paragraph are more in harmony with the observations of Moenkhaus and Godlewski.

CONCLUSIONS

The spermatozoon has two distinct effects upon the egg: namely, it causes its development and it transmits certain parental hereditary characters to the offspring. The experiments in heterogeneous hybridization confirm the idea supported by the experiments on artificial parthenogenesis, that the formation of the embryo is purely a matter of the egg and that the main function of the spermatozoon is the causation of the development of the egg. If we may express this statement in the form of a paradox we may say that fertilization is primarily and essentially artificial parthenogenesis. The transmission of hereditary characters through the sperm is in many cases merely an accessory function. It becomes of vital importance only in those forms where the male is heterozygous for sex and where the species can only be propagated through sexual reproduction.

If the sperm nucleus be chemically almost identical with the egg nucleus it is possible for it to force one or a few characters upon the developing embryo. If the difference between sperm and egg nucleus exceed a certain limit—which structural chemistry may one day be able to define—the hereditary influence of the spermatozoon is as a rule completely or almost completely obliterated; and the result is a purely maternal larva, rendered more or less sickly through the presence or formation of foreign or faulty substances.

The camera drawings of the sea-urchin larvae were made by Mr. W. O. R. King, those of the fish embryos by Mr. Bagg. To both gentlemen I wish to express my thanks.

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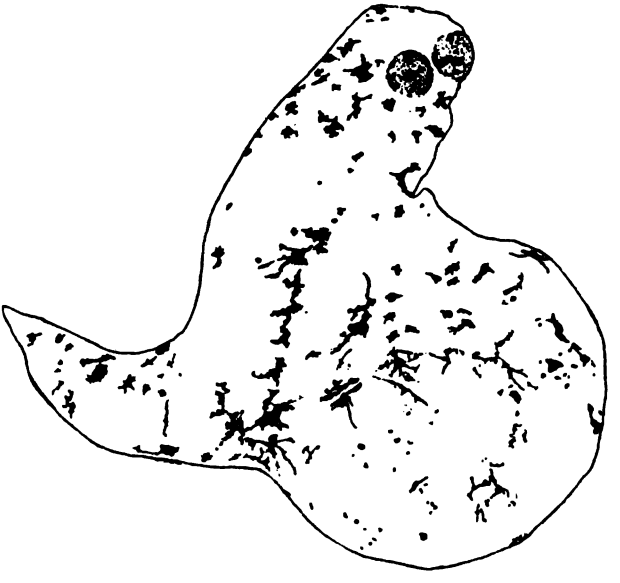


Fig. 16 Hybrid between Fundulus heteroclitus ♀ and Menidia ♂, thirty days old.

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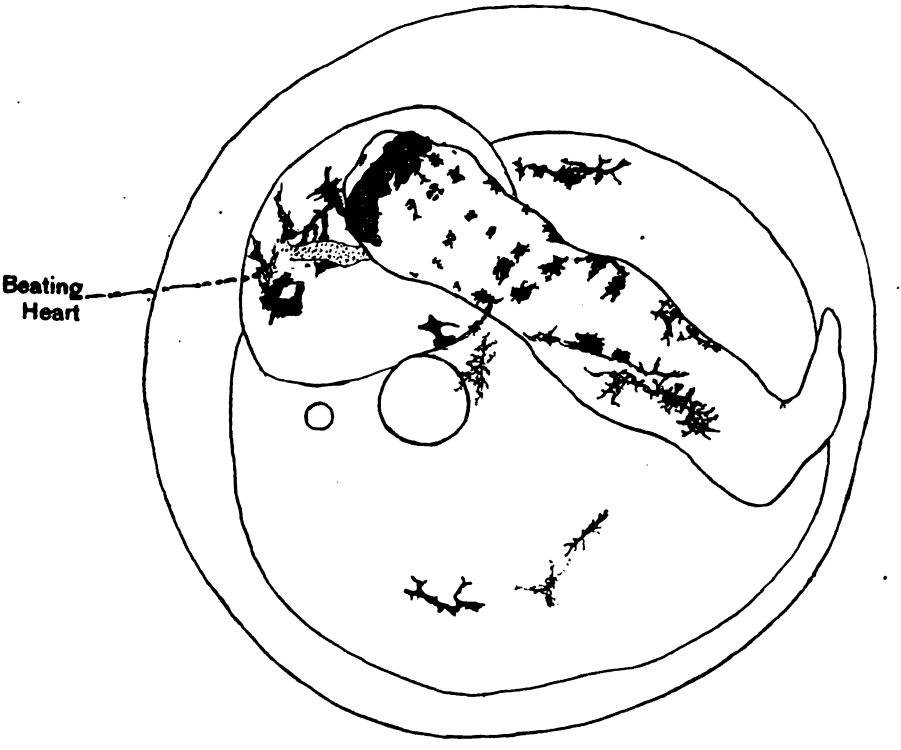


Fig. 17 Embryo of Fundulus heteroclitus thirty-one days old, raised in 50 cc. sea water + 2 cc. 0.01 per cent NaCN.

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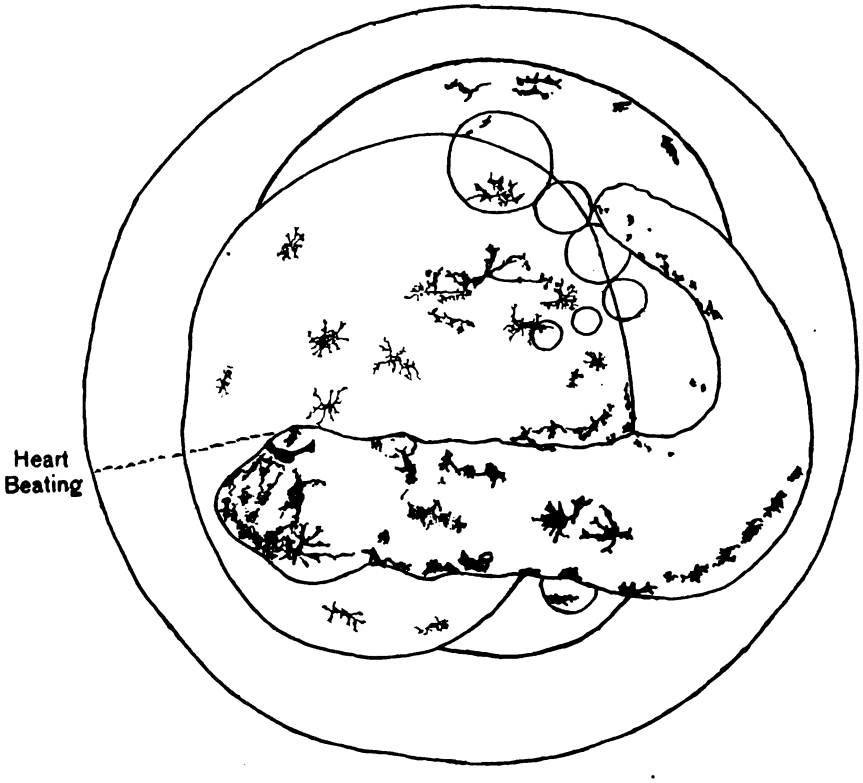


Fig. 18 Embryo of Fundulus heteroclitus thirty-one days old, raised in 50 cc. sea water + 2 cc. 0.01 per cent NaCN.

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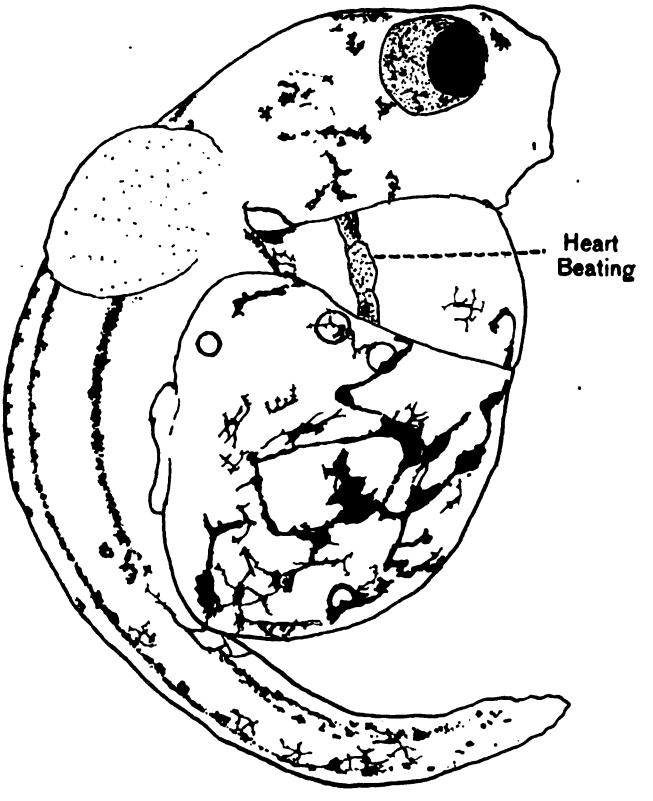


Fig. 19 Hybrid between Fundulus heteroclitus ♀ and scup ♂, twenty-five days old.

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TECHNIQUE AND REMOTE RESULTS OF VASCULAR ANASTOMOSES[1]

By ALEXIS CARREL, M. D., New York City
From the Laboratories of the Rockefeller Institute for Medical Research.

A. INTRODUCTION.

THE method of vascular anastomosis that I will describe to-night came into existence nine years ago from the study of the technique of Payr and of Murphy. Its principle was worked out on human cadavers and on two living dogs at the University of Lyons. It became then possible to perform successfully blood-vessels anastomosis. Afterwards, in the course of many experiments made at the University of Chicago, and, chiefly, at the Rockefeller Institute for Medical Research, the causes of all possible complications were analysed, and adequate procedures, developed in order to prevent their occurrence. With this technique improved and slightly modified in its details, a great many experimental operations were performed, and their results were observed after several years. The method was also used on human beings by several American and European surgeons. To-day its study can be considered as completed from both standpoints of the technique and the experimental results.

B. TECHNIQUE.

1. *General rules.* In operations on blood-vessels certain general rules must be followed. These rules have been adopted with the view to eliminate the complication which may especially occur after vascular sutures, namely, stenosis, hæmorrhage, and thrombosis.

A rigid asepsis is absolutely essential. Sutures of blood-vessels must never be performed in infected wounds. It seems that the degree of asepsis under which general surgical operations can safely be made may

[1] Read before the Clinical Congress of Surgeons of North America, Philadelphia, November 9, 1911.

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be insufficient for the success of a vascular operation. It is probable that a slight non-suppurative infection, which does not prevent the union of tissues, "per primam intentionem," may be sufficient to cause thrombosis.

Thrombosis also follows injuries of the wall of the vessel. The arteries and the veins can be freely handled with the fingers without being injured, provided the wall is not crushed with forceps or other instruments. If a forceps be used, it must take between its jaws nothing but the external sheath. When temporary hæmostasis is obtained by means of forceps or clamps, these forceps or clamps must be smooth-jawed and the pressure of the jaws carefully regulated.

The dessication of the endothelium may also lead to the formation of a thrombus. During the operation the wall of the vessels must be humidified with Ringer's solution, or covered with vaseline. The presence of coagulated blood, of fibrin ferment or of foreign tissues or tissue juices on the interior can determine the production of a thrombus. It is, therefore, necessary to resect or to remove the external sheath from the edges of the vessels. For, if during the suture, it gets between the edges of the vessels, or around the silk threads, it very quickly leads to a deposit of fibrin, and possibly to an obliterative thrombus. By washing carefully the vessel with Ringer's solution and coating it and the surrounding parts of the operating field with vaseline, we can efficiently protect the endothelium against coagulating blood and juices of tissues. Vaseline prevents, also, the fingers of the operators and the threads from being soiled by fibrin ferment.

As perforating stitches are always used, the endothelial layer is necessarily wounded by the needle. These wounds are rendered as harmless as possible by the use of very fine and sharp, round needles. Extremely small wounds are made. The threads are sterilized in vaseline and kept heavily coated with it during the suture. Vaseline is rubbed off

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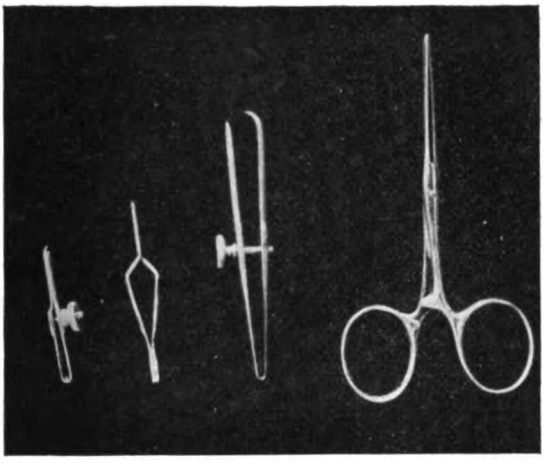


Fig. 1. Small Crile forceps. Ordinary serrefine. Crile forceps. Elastic-jawed forceps.

in the holes, covers the silk threads, and prevents the wounded tissue and the foreign material from coming into actual contact with the blood.

Stenosis is very liable to occur at the point of anastomosis. Therefore, the suture must be performed while the wall is under tension. The tension can easily be obtained by traction on retaining stitches properly located. Great care is taken to obtain a smooth union and approximation of the internal coats. When these general rules are observed, the operation can be safely performed.

2. *Instruments.* Temporary hæmostasis is secured by Crile clamps or by elastic-jawed forceps (Fig. 1), when large vessels, like the aorta of a dog or the femoral artery of a man, are sutured. On smaller vessels, we use a small Crile clamp, made by Collins, or an ordinary serrefine (Fig. 1). A serrefine, the spring of which is readily regulated, is the more convenient instrument for temporary hæmostasis. These forceps are smooth-jawed and are used without rubber. In the large vessels, the circulation can also be stopped by a narrow rubber strip which is drawn tight around the vessel and fixed by a strong serrefine or foreceps. When the circulation

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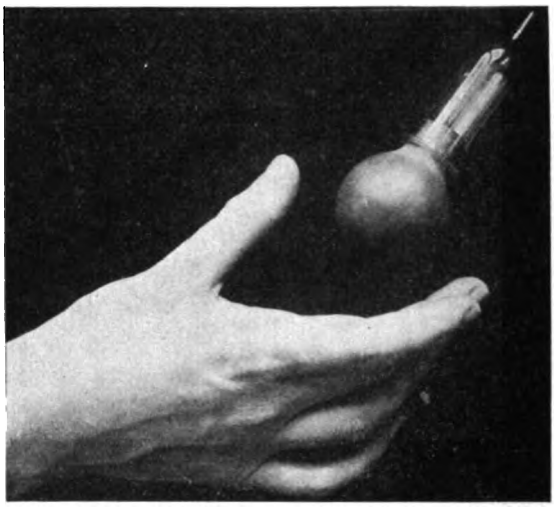


Fig. 2. Gentile's syringe.

cannot be interrupted, intubation of the vessel is performed. A paraffined tube is introduced into its lumen, and fixed at each end by a rubber band. Then it becomes possible to resect the wall without hæmorrhage while the circulation goes on through the lumen of the tube.

Washing of the vessels is made with a Gentile syringe (Fig. 2), which is composed of a glass and of a rubber bulb. The fluid used is Ringer's solution.

The needles are round, straight needles, Kirby No. 16 (Fig. 3), for the small vessels, and even for the large vessels. A human femoral artery can be sutured with a needle No. 16. In that case, needle No. 12 can also be used. The needles are handled with the fingers, without needle-holders. There are also short, curved needles, which are easily handled by a Collins needle-holder. I have never used them. But they could be employed in some cases. The silk threads are very fine and sterilized in vaseline. The needles are threaded and the thread is rolled on card board. Then, needles and threads are placed in a hermetically closed jar, which is put in the sterilizer under fifteen or eighteen pounds pressure. The needle are removed from the

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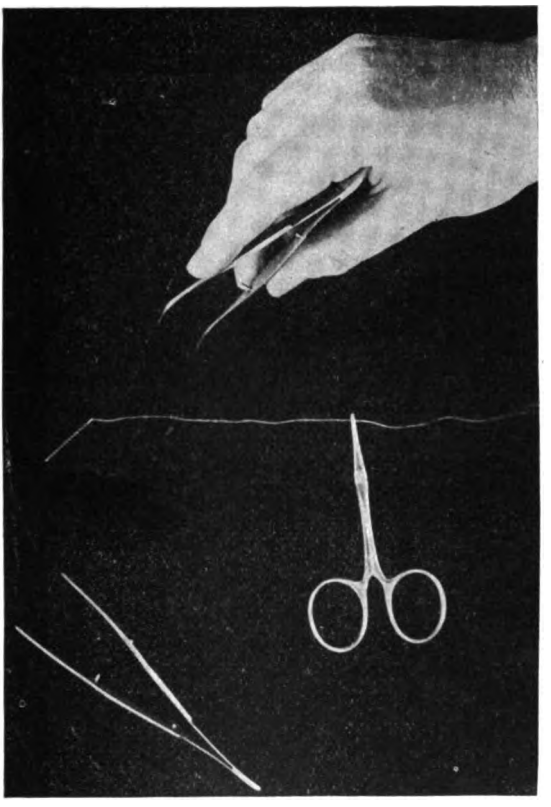


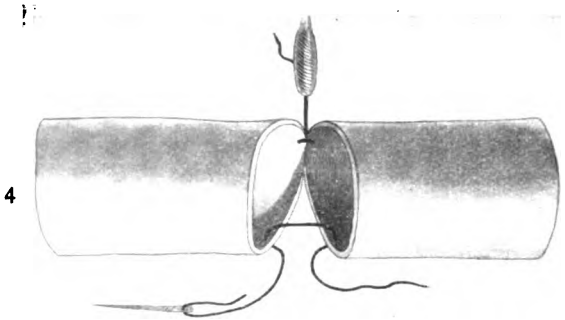
Fig. 3. Dissecting forceps. Gentile's small forceps. Needle No. 16.

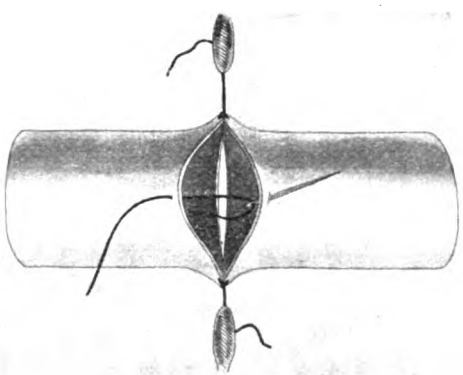
jar when the vaseline is still warm and deposited on a black towel.

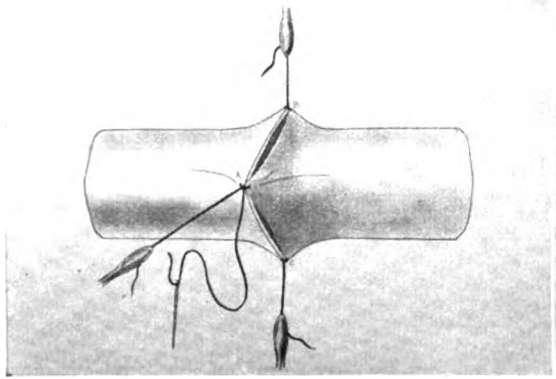
The ends of the threads are fixed during the suture by very small Gentile hemostatic forceps (Fig. 3). The operative field is circumscribed by a black Japanese silk towel on which the fine threads can easily be seen (Fig. 3).

3. Temporary hæmostasis and preparation of the vessels. The vessels are exposed by a large incision and they are freely dissected. The close dissection of a very long segment of artery or vein is not dangerous and it is important that the operating field is large. Careful hæmostasis of the wound is made because during the suture the operating field must be free from blood. The temporary

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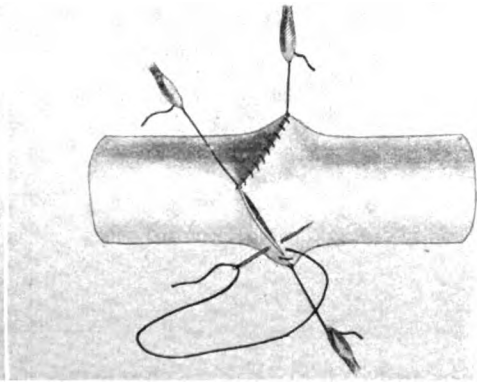


Fig. 4. Arterial anastomosis. Application of the two posterior retaining stitches.
Fig. 5. Arterial anastomosis. Application of the anterior retaining stitch.
Fig. 6. Arterial anastomosis. Circumference of artery transformed in a triangle by traction on the retaining stitches.
Fig. 7. Arterial anastomosis. Continuous suture.

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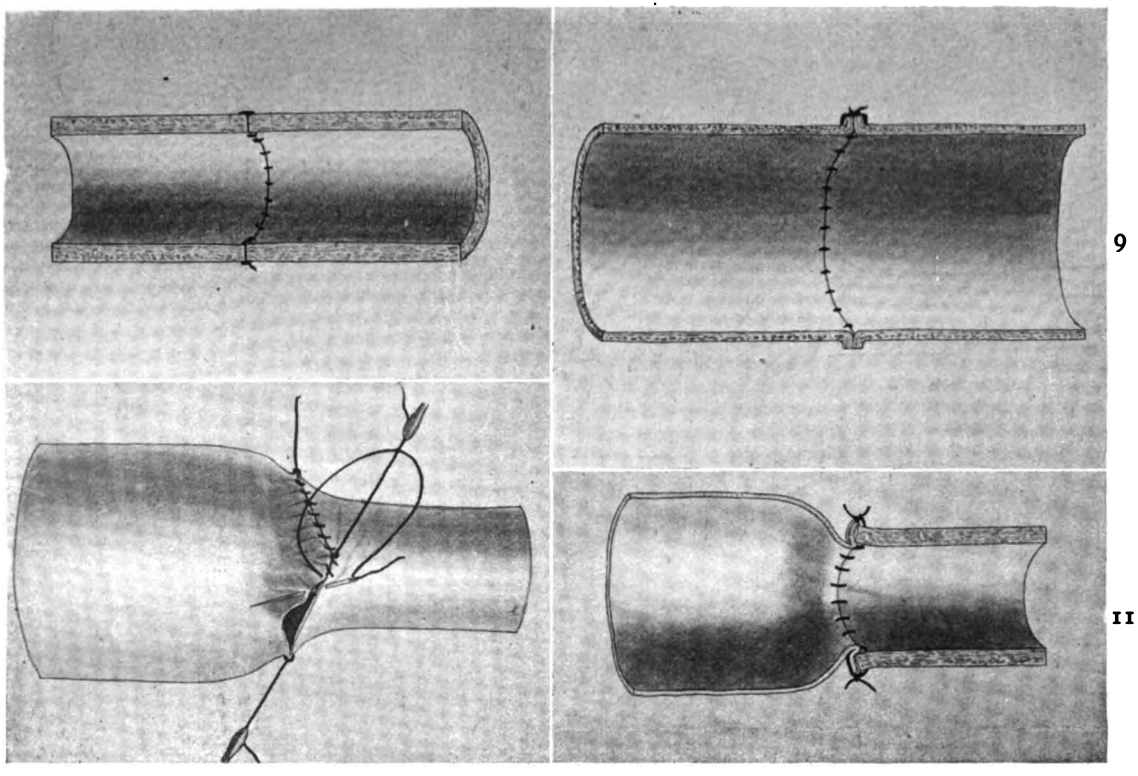


Fig. 8. Arterial suture. Approximation of the ends.
Fig. 9. Venous suture. Approximation of the ends.
Fig. 10. Arteriovenous anastomosis. Continuous suture.
Fig. 11. Arteriovenous suture. Approximation of the ends.

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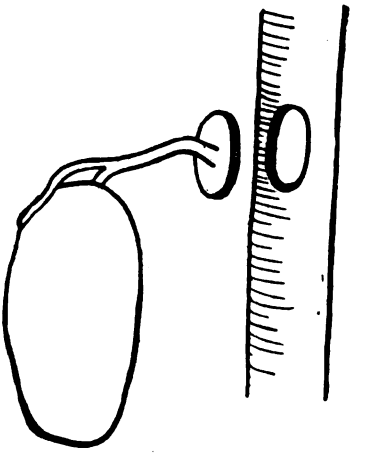


Fig. 12. Termino-lateral anastomosis.

hæmostasis is secured by clamps, forceps, or rubber bands, placed at a few centimeters from the location of the future anastomosis. If there are collaterals between the forceps and the point of section they are ligated or clamped.

Then the vessels are cut and the external sheath is resected or removed from the ends of the vessels.

The end of the syringe is introduced into the lumen of the vessels. The blood is washed out not only from the vessels but also from the operating field. The fluid is removed with dry gauze sponges. Then the vessels and the surrounding parts are covered with warm vaseline. Around the ends of the vessels is placed a black Japanese silk towel.

4. *Suture.* (*a*) *The termino-terminal anastomosis* consists of uniting the ends of two vessels by a continuous suture. Arterial, venous and arteriovenous anastomoses will be described.

In the arterial anastomoses the ends of the arteries must come into contact without strong traction. They are united by three retaining stitches located in three equidistant points of their circumference. The needle perforates the arterial walls at a short distance from the edge and a first posterior thread is placed and tied (Figs. 4 and 13). One end

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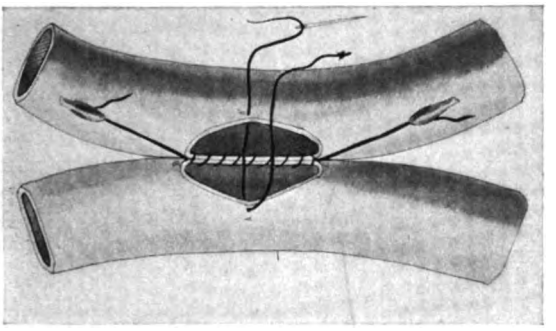


Fig. 13. Latero-lateral anastomosis. Posterior line of suture. Application of the anterior retaining stitch.

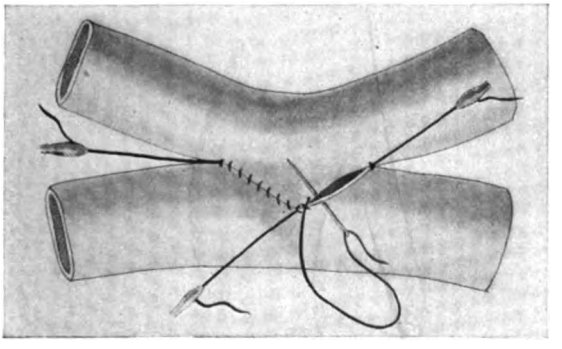


Fig. 14. Traction on the retaining stitches and anterior continuous suture.

of the thread is fixed with a small forceps, while the other end with the needle is laid on the black towel and will be used for the continuous suture.

Then the second posterior retaining stitch is placed, and both ends of the thread are fixed by a forceps (Fig. 5). A slight traction is made on both posterior threads. It is very easy to place the anterior retaining stitch (Figs. 5 and 16). Then the ends of the arteries are united in three equidistant points by the three stitches. By traction on the threads, the circumference of the arteries can be transformed in a triangle (Fig. 17).

The edges of each side of the triangle are united by a continuous suture made with the posterior needle. The first side of the triangle is placed under tension by traction

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Fig. 15. Arterial suture. Application of the first posterior retaining stitch.

on the forceps and its edges are sutured. The edges of the second and the third sides are united in the same manner (Figs. 7 and 17). A few stitches only are necessary. In the case of an artery of the size of the carotid of a middle-sized dog, three stitches are made on each side of the triangle. During the suture, great care is taken to approximate exactly the surfaces of section of the wall (Fig. 8). The raw surface must not come in contact with the blood stream. Before the complete losing of the artery, vaseline is removed by pressure from its lumen.

In venous anastomoses the ends of the veins are also united by three retaining stitches. A venous suture requires more stitches than

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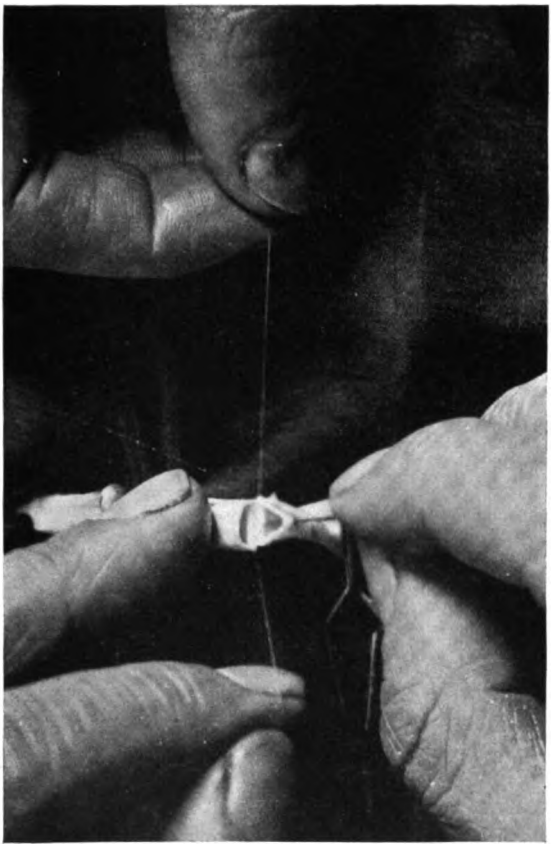


Fig. 16. Traction on the two posterior retaining stitches.

an arterial suture, on account of the thinness of the wall. The approximation is made by eversion of the edges, which are united not by their surface of section, but by their endothelial surface (Fig. 9). The inversion of the edges is very defective and may bring about the formation of a thrombus.

In arteries venous anastomoses, the vein is generally larger than the artery. After the ends of both vessels have been united by the three retaining stitches, each side of the venous triangle is longer than the corresponding side of the artery. Then a traction is made on the retaining stitches. The arterial end can be very much dilated and brought nearer to the size of the vein.

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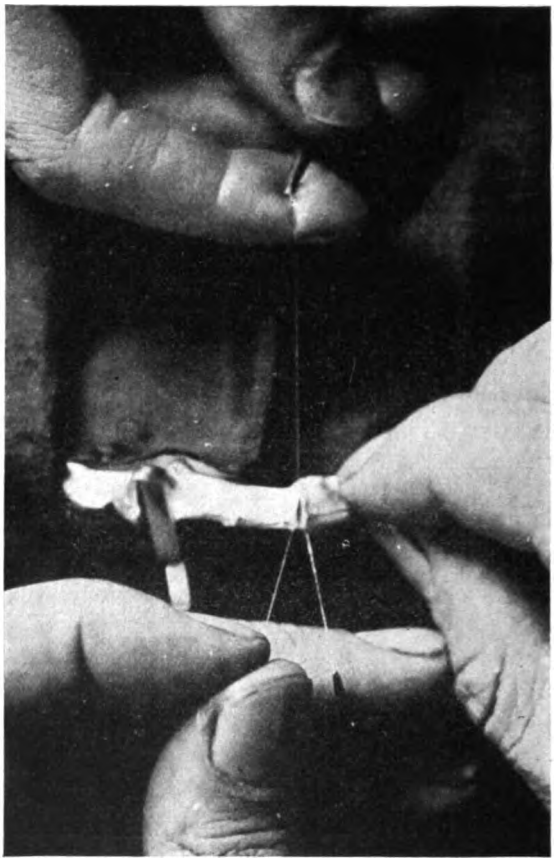


Fig. 17. Continuous suture.

Each stitch of the continuous suture is made larger on the vein than on the artery (Fig. 10). The caliber of the vein is progressively reduced and a good union takes place. The venous wall is turned outward and its endothelial surface is applied against the surface of section of the arterial wall (Fig. 11).

(*b*) *The termino-lateral anastomosis* consists of implanting the end of a vessel onto the wall of another vessel. A triangular or elliptic opening is made through the wall of one vessel. The edges of the opening are approximated by three or four retaining stitches to the end of the other vessel, and united by a continuous suture. When the vessel is of a small caliber, it is better to

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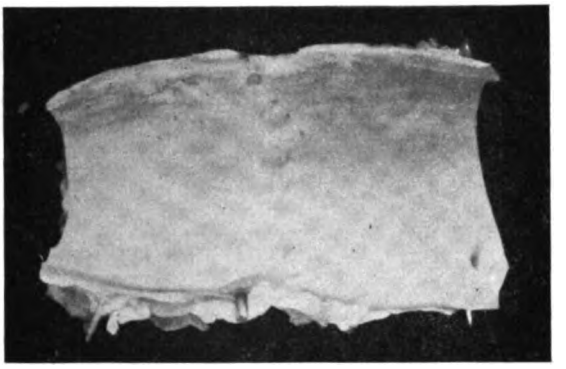


Fig. 18. Anastomosis of the aorta.



Fig. 19. Ten months after the operation. Location of the anastomosis marked by black crosses.

perform the termino-lateral anastomosis by the patching method. It consists in dissecting the small artery to the level of a larger trunk, and in resecting the patch of the wall surrounding the mouth of the small artery. The patch is elliptic in its shape, and the edges as regular as possible in order that a smooth anastomosis can be made. Then the patch is inserted into an opening made through the wall of the larger vessel, and fixed by four retaining stitches and a continuous suture. By this method, the termino-lateral anastomosis is more safely performed than by the direct implantation of the end of the smaller vessel onto the wall of the larger.

(*c*) *Latero-lateral anastomoses*. Both vessels are placed near one another and opened

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Fig. 20. Arteriovenous anastomosis. Fifteen months after the operation.



Fig. 21. Arteriovenous anastomosis. Twenty months after the operation.

longitudinally by incision, or resection of an elliptic flap. The ends of the openings are united by a first posterior retaining stitch. A second posterior retaining stitch unites also the other ends of the incisions, or two points located on the internal edges of the wounds at a very short distance from the end. The ends of the retaining stitches are, of course, on the external side of the vessels. Then, the needle perforates the wall and is drawn inside of the vessel. A traction is made on the retaining stitches and a continuous suture is made on the endothelial side of the vessels (Fig. 13). At the other end the needle perforates again the wall, and the suturing thread is tied on the external side of the vessel to the retaining thread. While this posterior line of suture is stretched by traction on the posterior retaining thread, an anterior retaining stitch unites two points

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Fig. 22. Two years after section of the thoracic aorta and graft of a segment of vein preserved in cold storage.

of the free edges (Fig. 13). By traction on the posterior and anterior retaining stitches the openings are transformed in a triangle and the union is completed by a continuous suture running along both anterior sides (Fig. 14).

5. *Re-establishment of the circulation.* Before the circulation is re-established, the line of sutures is carefully examined, and if a gap is found between two stitches, it is immediately closed. Then, gauze sponges are placed on the lines of suture, and the clamps are removed, while a gentle pressure is made on the sponges. There is almost always some leakage during the first minutes. After two of three minutes, the sponges are removed, and if some hæmorrhage persists, one or two complementary stitches are added.

Then, the vessels and the operating field are washed with Ringer's solution, and the wound closed without drainage.

C. RESULTS.

A great many anastomoses have been performed with this method. If the technique here described is followed, no complications occur. The results are practically

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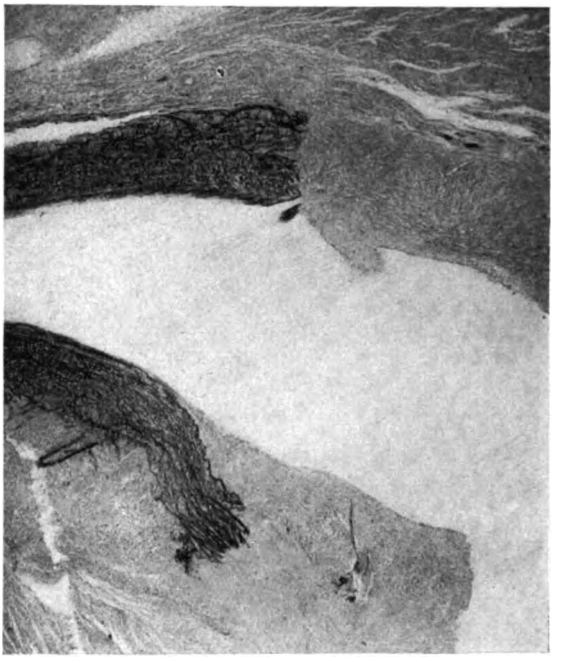


Fig. 23. Section of an arteriovenous anastomosis. Weigert elastic tissue stain.

always positive. Therefore, it has permitted the solution, from a surgical standpoint, of important problems, such as transplantation of venous segments of arteries, homoplastic and heteroplastic transplantations of blood-vessels, transplantation of vessels in latent life, of devitalized vessels, of pieces of veins, peritoneum and rubber on arteries, replantation and transplantation of spleen, thyroid gland, kidneys, limbs, etc. Many animals were kept alive for a long time. Therefore, the anatomical and clinical results of the anastomoses are completely known.

Immediately after the operation the endothelial coats of the vessels appear as closely approximated and the stitches are seen in the lumen of the vessel. After eight days, the appearance is slightly modified; the line of union is less apparent. The threads are uncovered by fibrin (Fig. 18). The photograph of an arteriovenous anastomosis taken fifteen days after the operation, shows

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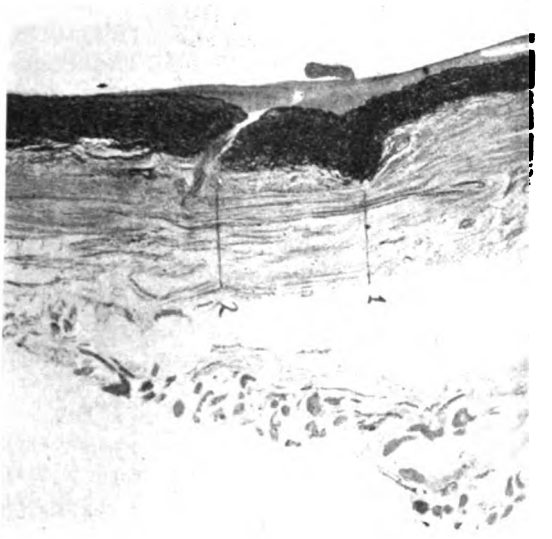


Fig. 24. Transplantation of the aorta. Weigert elastic tissue stain. 1. Anastomosis. Union of the medias without scar. 2. A stitch.

the appearance of the scar when the stitches are very regularly disposed. In the arterial anastomosis, the stitches disappear progressively and the scar becomes very small. Sometimes it is slightly depressed. No stenosis was ever observed. After six months, the stitches have completely disappeared on an arterial suture, while on the venous anastomosis of a replanted kidney, the threads could be seen ten months after the operation (Fig. 19). The anastomosis of the artery (Fig. 19) of the same kidney had become, after ten months, completely invisible. On arteriovenous anastomosis, fifteen and twenty months after the operation (Figs. 20 and 21), the location of the anastomoses can be determined on account of the difference of caliber. But it is perfectly smooth and there is no dilatation or stenosis at its level. After the extirpation and replantation of the spleen with circular suture of the splenic vessels, the caliber of the

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artery and of the vein remains entirely normal after twenty-two months. On the photograph, the location of the anastomosis is indicated by two whitish lines (Fig. 22). Several other animals have lived, or are still living, two years, two years and a half, three years, and four years after having undergone several kinds of vascular anastomosis. The results are uniformly excellent.

The study of the sections of the anastomoses shows that the union of the ends of the vessels is always very accurate (Fig. 23), and can even take place without scar (Fig. 24). But such a perfect result is exceptional. In no case was dilatation or stenosis at the level of the line of sutures observed. The technique, therefore, brings about an almost perfect reconstruction of the vessel. It explains the constancy of its clinical results.

The technique has been used for several years on human beings. Crile employed it at the beginning of his method of blood transfusion. Many other American and European surgeons performed anastomoses of large vessels. Even the aorta of a child could be successfully anastomosed by Braun. The blood-vessel operations are easier on human beings than on dogs, and it seems that the results are as satisfactory.

It can be concluded that this simple method of anastomosing blood-vessels gives results that remain excellent after a long time, and that it can be used safely on human beings, provided the rules of the technique be strictly followed.

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ULTIMATE RESULTS OF AORTIC TRANSPLANTATIONS.*

BY ALEXIS CARREL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATE 58.

The purpose of this article is to describe the ultimate result of the transplantation of vascular segments upon the thoracic and abdominal aortas. It is known that a severed artery can be efficiently repaired by segments of autogenic, homogenic, or heterogenic vessels in a condition of active or latent life, by devitalized arteries, by pieces of peritoneum, and even by pieces of rubber. It is now important to ascertain whether or not these repairs are lasting.

In several cats and dogs in which portions of the abdominal or thoracic aortas have been replaced by segments from other vessels, I have observed a normal circulation after two, three, and four years. As two of the animals died a few months ago, the anatomical results of the transplantations could be examined.

Experiment 1. Transplantation of a Human Artery upon the Abdominal Aorta of a Dog.—A young, small sized black bitch was used. On May 6, 1907, under ether anesthesia, a transverse semicircular laparotomy was made which was followed by a dissection of the abdominal aorta below the renal arteries, and ligation of four collateral lumbar branches. During temporary hemostasis, the aorta was cut and a short segment resected, a segment of the upper part of a human popliteal artery being interposed between the cut ends of the aorta. This segment was 2.5 centimeters long, and had a diameter which was more than one third greater than that of the aorta. It was normal in appearance. Nevertheless, the wall had partially lost its elasticity. The vascular ends were sutured by the ordinary method, the circulation was reëstablished, and the abdominal wall was closed by four planes of sutures.

The segment had been extirpated on April 11, 1907, from a limb amputated on April 10 at the Presbyterian Hospital by Dr. Ellsworth Eliot. The limb was from a young man who had an osteosarcoma. Through the kindness of Dr. Eliot, the leg was put in the ice box on April 10, and sent to the Rockefeller Institute on April 11, 1907. The popliteal artery and the lower part of the

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femoral artery were extirpated. Before being used in this experiment, the upper part of the popliteal artery had been put in isotonic salt solution, and preserved for twenty-four days in a refrigerator at a temperature slightly above the freezing point.

Following the operation, the femoral pulse of the dog was normal and the animal remained in good health.

October 18, 1907. The animal was in excellent health. A median laparotomy under anesthesia was made, and the pulsation of the abdominal aorta was found to be normal. There was no dilatation of the inserted segment, which was very much larger than the aorta, and no change in the consistency of its wall.

During the years 1908, 1909, 1910, and 1911, the animal remained in excellent health and became very fat. She was pregnant several times, and had normal pups. In June, 1911, the animal died during labor, and the body was preserved in formalin until it could be carefully examined.

Autopsy.—In October, 1911, the lower part of the body was examined. The peritoneal cavity and the abdominal organs were normal. The abdominal aorta was dissected and removed.

Macroscopical Examination.—In the middle portion of the abdominal aorta, a dilated segment was seen (figure 1). The dilatation was not very much greater than at the time of the transplantation. On opening the aorta and the transplanted segment longitudinally, the anastomoses were easily seen. The length of the segment was 2 centimeters. From above downward, the circumferences of the artery and segment at their anastomoses were as follows: upper part of artery, 11 mm.; upper part of segment, 12 mm.; lower part of segment, 10 mm.; lower part of artery, 8 mm. The circumference of the middle part of the transplanted segment was 20 mm. The scar of the upper anastomosis was linear, while that of the lower anastomosis was thicker. The internal surface of the segment was white and glistening, but just below the upper anastomosis, this surface had a cicatricial appearance. The wall of the transplanted segment was generally thicker than that of the aorta. Its consistency was harder, and in certain regions it seemed calcified.

Microscopical Examination.—The vessel wall was cut transversely and the sections were stained by hematoxylin and eosin, by Van Gieson's method, and with Weigert's elastic tissue stain. The wall was composed exclusively of connective tissue, which, in a few places, showed hyaline degeneration. No elastic fibers or muscle cells were seen.

Experiment 2. Transplantation of a Segment of a Dog's Vein upon the Thoracic Aorta of a Dog.—On November 5, 1909, a white female fox terrier was etherized by the Meltzer-Auer method, and a left transverse thoracotomy was followed by dissection of the descending aorta and ligation of four collateral branches. During a temporary hemostasis, a section of the thoracic aorta was removed and between the cut ends was interposed a segment of the external jugular vein extirpated from a large dog on November 4, and kept for twenty-four hours in cold storage. This segment was sutured to the ends of the aorta by the ordinary method, and the circulation was reëstablished after an interruption of seventeen minutes. The thoracic cavity was closed by four planes of sutures.

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November 7. In the afternoon, the animal was in excellent condition, but the posterior limbs were stiff and hyperextended. The dog could stand up, although she was not able to walk. The femoral pulse was normal.

November 9. The animal walked, but the posterior limbs were still in hyperextension.

November 14. The condition of the dog was normal, and the animal could run, although the posterior legs were still stiff.

In May, 1910, the animal was sent to the farm, but was brought back to the Institute in October, 1910. She became pregnant and had several pups in October, 1911.

On January 10, 1912, the animal was infected with myelitis which was epidemic. She became paralyzed and died on January 16.

Autopsy.—The thoracic and abdominal organs were in normal condition. The posterior part of the left lung was slightly adherent to the thoracic wall. The lung was normal. On dissecting the aorta, no evidence of the previous operation was seen. Nevertheless, the location of the transplanted segment was detected by a slight increase in the diameter of the aorta. The descending aorta and the transplanted segment were then extirpated and opened longitudinally.

Macroscopical Examination.—The transplanted segment was about 4.5 centimeters below the arch of the aorta. It was 2.5 centimeters long and its diameter was slightly larger than that of the aorta (figure 2). Its internal surface was white and glistening and resembled the internal surface of the aorta. Two valves of the jugular vein had persisted and presented the same appearance as at the time of the transplantation (figure 3). The anastomoses of the artery and the transplanted segment were almost invisible, as at the place of juncture neither contraction nor dilatation had occurred; but the wall of the transplanted segment was thinner, more translucent, and harder than that of the aorta.

Microscopical Examination.—A small piece of the middle part of the transplanted segment was cut and stained with hematoxylin and eosin, with Van Gieson's and with Weigert's stains. The wall was apparently composed of a normal adventitia and of a media in which neither elastic fibers nor muscle cells could be seen. On its internal surface, endothelial cells were observed.

SUMMARY.

In the first experiment, a segment of popliteal artery, extirpated from the leg of a young man and preserved for twenty-four days in cold storage, was transplanted upon the abdominal aorta of a small bitch. The animal lived in excellent health for four years and two months, became pregnant several times, and finally died during labor. The abdominal aorta was normal, but the human arterial segment was slightly dilated, and its wall was composed of connective tissue only.

In the second experiment, a segment of dog's jugular vein, pre-

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served for twenty-four hours in cold storage, was transplanted upon the thoracic aorta of a fox terrier. In spite of slight lesions of the cord due to interruption of the aortic circulation during the operation, the animal remained in excellent health. After two years and two months, the dog died of an epidemic disease. The descending aorta was normal. The transplanted segment had about the same caliber as that of the aorta, but its wall was composed of connective tissue, with no evidence of muscular or elastic tissues.

In these two experiments, the walls of the transplanted segments contained neither muscle nor elastic tissue fibers and were not thicker than those of the artery. Nevertheless, they were able to withstand the pressure of the blood without undergoing any marked dilatation.

If death due to accident had not brought these two experiments to an end, it is probable that the transplanted segment would have successfully resisted the pressure of the blood for a much longer time. As it is, the result shows that, in one experiment after four years and in the other after two years, the segments transplanted upon the abdominal and thoracic aortas were still efficient.

EXPLANATION OF PLATE 58.

FIG. 1. The transplanted arterial segment and the adjacent part of the aorta.
FIG. 2. The descending aorta with the transplanted venous segment.
FIG. 3. The transplanted venous segment and its valves.

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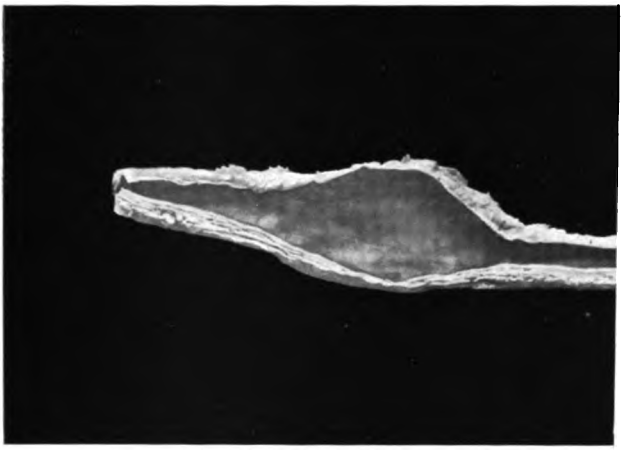


FIG. 1.



FIG. 2.



FIG. 3.

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TECHNIQUE FOR CULTIVATING A LARGE QUANTITY OF TISSUE.*

BY ALEXIS CARREL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

In a previous article,[1] a technique for cultivating large quantities of tissue was briefly described, but the results given by this method were inconstant, and by it the growth could not be watched under the microscope. Moreover, the fluids which exuded from the culture fell into and mixed with the water covering the bottom of the box, aeration was difficult, and bacterial infection often occurred. In order that we might study the functions of growing tissues, it was necessary to improve the method. The procedure which we now employ in the preparation of the tissues, the medium, and the cultures, and the method of examining the cultures will be described in this article.

1. *Preparation of the Tissues.*—By means of a sharp instrument, the tissues are divided into very small fragments in Ringer's solution, care being taken not to crush the tissues. The tissues of chicken fetuses can be easily reduced without crushing to fragments measuring about 0.5 or 0.1 of a millimeter in diameter. More friable tissues, dog's thyroid, for example, cannot be cut so finely. The fragments are suspended in Ringer's solution, and are then taken up in a large pipette. Each drop of the fluid contains approximately the same number of fragments. By depositing the same quantity of fluid on each plate, we obtain cultures which contain practically the same quantity of tissue.

2. *Preparation of the Medium.*—The best culture medium is plasma diluted with about two fifths of its volume of distilled water. Serum mixed with one fifth of its volume of a 2 per cent. agar

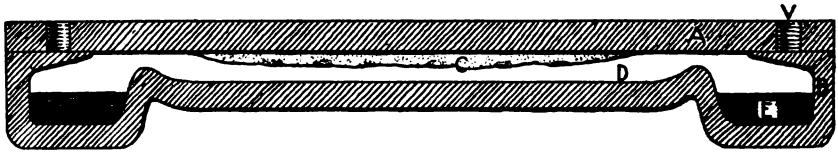
* Received for publication, February 16, 1912.

[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

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solution can also be used, but the growth is less abundant than in plasma. When plasma is employed in the preparation of cultures which have been previously washed in Ringer's solution, it coagulates very slowly. Complications, such as concentration of the medium by evaporation, and bacterial infection, may then occur. In order to accelerate the coagulation of the plasma in these cases, muscle, or embryonal extracts, or serum is added.

3. *Preparation of the Cultures.*—The cultures are prepared in Gabritschewski boxes or in larger glass boxes. A Gabritschewski box consists of a flat, circular glass dish covered by a circular glass plate (text-figure 1). The dish measures 110 millimeters in diam-



TEXT-FIG. 1. Cross-section of a Gabritschewski box. A represents the cover; B, the box; C, the culture; D, the lower inner surface; E, the groove filled with water; V, the hole.

eter, and is 12 millimeters in height. Around the periphery of the dish, at the bottom, runs a groove which contains water, while the fluids exuded from the culture are retained in the central depression which measures 65 millimeters in diameter. The cover is sealed to the circumference of the dish by vaselin. At the ends of the same diameter and near the edge of the cover are two holes. When the cover is in place, the atmosphere of the box is isolated from the external atmosphere unless the cover is rotated so as to bring its two holes in apposition with two similar holes in the upper edge of the dish. The culture medium and the fragments of tissue are distributed evenly over the lower surface of the plate, and may cover an area 65 millimeters in diameter. After the medium hardens, the tissues adhere to the plate and this can be turned over and sealed to the dish.

The larger boxes consist of deep circular dishes covered by glass plates. These boxes, as well as the Gabritschewski boxes, are sterilized by dry heat. The bottom of each dish is covered with water.

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A large Petri dish, fixed on a stand, receives the fluid which exudes from the culture. The cover is sealed to the dish with vaselin. The culture on the under surface of the plate may cover an area about 150 millimeters in diameter.

When the culture is to be washed, the tissues are not placed directly on the covers, but are first deposited on a silk veil which is stretched upon a rectangular glass frame having an area of about 14,000 square millimeters.

A culture on a silk veil is prepared in the following way: The glass frame on which the silk is stretched is placed on the glass cover and is fixed to it by paraffin. Then the silk is moistened with Ringer's solution, or with plasma diluted with distilled water. The tissues in Ringer's solution are then deposited on the cover by means of a large pipette, and are so spread that the fragments are evenly distributed and are separated by free spaces. When the fragments are crowded in a small area, they do not grow abundantly and their life is short. Then a quantity of plasma is added that is about three times as large as that of the suspension of tissue. If necessary, a few drops of serum or of muscle extract are also added. During the coagulation of the medium, the plate is protected from atmospheric dust. As soon as the medium has become sufficiently hard, the plate is placed on the box, which is closed hermetically by vaselin.

When, after a few days, the culture has to be washed, the cover is placed above a jar filled with cold Ringer's solution. The frame is detached with a knife and deposited in the fluid. After one or two hours, it is removed from the Ringer's solution, placed on another cover, fixed with paraffin, and covered by a new medium. By this procedure, rejuvenation of large cultures can be brought about,[2] but bacterial infection frequently occurs.

4. *Examination of the Cultures.*—The boxes are incubated at a temperature adapted to the nature of the tissues, generally 38° or 39° C. The tissues grow as extensively as in small cultures in a hanging drop. The growth can be watched without the help of a microscope. After one or two days, the outline of the fragments

[2] Carrel, *Jour. Am. Med. Assn.*, 1911, lvii, 1611.

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becomes less sharp, and is surrounded by a narrow opalin area. The size of the fragment increases. When the tissues are growing rapidly, a little fluid exudes from the medium and collects on the under surface of the tissue. Simultaneously the tension of the atmosphere in the box diminishes. If there is bacterial infection, the colonies are easily recognized by their opacity and by their sharp outline.

The cultures in the Gabritschewski boxes may be examined microscopically, although a high power cannot be used on account of the thickness of the cover. If minute morphological examination of the tissues is required, the cultures are washed for one hour in Ringer's solution at 0° C. and are then fixed in isotonic sodium chlorid solution containing 2 per cent. formalin. They are stained afterwards without being detached from the glass plate. If the medium is very thick, the culture, after having been fixed, is removed from the plate and divided into fragments which are embedded in paraffin and cut in serial sections.

When the cultures are to be inoculated into animals, the fragments of tissue and the surrounding cells are removed, washed in Ringer's solution, cut into small pieces, and injected under the skin.

For the study of the substances that develop in the medium during the life of the tissues, the culture is cut into small fragments, which, together with the exuded fluid, are taken up in a large pipette, placed in a centrifuge tube, and centrifugalized. The substances produced by the tissues may be studied in the supernatant fluid.

This technique for cultivating a large quantity of tissue is still far from perfect, and will be modified in the future. Nevertheless, by means of it, new problems may be studied.[8]

[8] Carrel and Ingebrigtsen, *Jour. Exper. Med.*, 1912, xv, 287.

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THE INFLUENCE OF HEAT ON DIFFERENT SERA AS CULTURE MEDIA FOR GROWING TISSUES.*

BY RAGNVALD INGEBRIGTSEN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 59–61.

In the cultivation of tissues outside of the organism, heterogenic plasma has proved a useful culture medium. In it, cells of mammals grow rather rapidly for several days at least. Carrel and Burrows[1] have shown that embryonic chick tissues develop extensively in plasma from rabbits, dogs, and human beings. Lambert and Hanes,[2] who cultivated rat sarcoma in heterogenic media, were able to keep this tissue alive in guinea pig plasma for thirty days by transferring it to fresh medium every seventh day. Of the media examined by them, only goat plasma proved unsuitable as a culture medium. In goat plasma no growth occurred. All these authors emphasize the fact that the growth of tissue is more rapid and extensive in homogenic than in heterogenic media.

In order to understand more thoroughly the laws governing life and growth of tissues outside of the organism, and possibly to find an explanation of the variations in growth in different media, it seemed to me to be of importance (1) to make an exact comparative investigation of the quantitative growth in autogenic, homogenic, and heterogenic media, and (2) to investigate the growth in heated as well as in unheated media. I have begun a study of these problems, and the present paper gives a preliminary report of my results.

In this series of experiments, I have used as a culture medium a mixture of serum and 2 per cent. agar.[3] Serum was chosen instead

* Received for publication, February 20, 1912.

[1] *Jour. Exper. Med.*, 1911, xiv, 244.

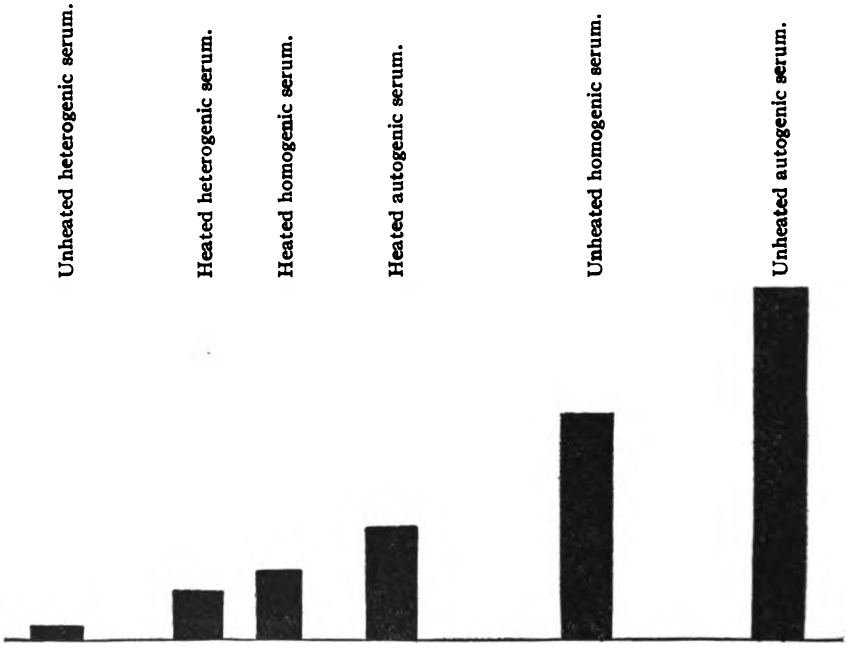
[2] *Jour. Exper. Med.*, 1911, xiv, 129.

[3] Fluid serum has been used as a culture medium by Carrel and Burrows (*loc. cit.*), who applied fine cotton threads as a scaffold for the growing cells. They found homogenic as well as heterogenic serum a useful culture medium for embryonic chick tissue.

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of plasma, in order that the medium might be exposed to heat without altering its physical qualities. Heating the medium exerts a marked influence on it, as will be shown by the results of this investigation.

Technique.—A 2 per cent. solution of agar in distilled water was kept fluid in a water bath at 60° to 65° C. It was quickly taken up in a pipette, and when the temperature had fallen to 38° to 40° C., it was thoroughly mixed with serum in the proper proportions. The



TEXT-FIG. 1. A comparison of the average growth of guinea pig bone marrow in different media after twenty-four hours.

mixture of serum and agar was then placed upon the piece of tissue on the cover-glass where it coagulated, forming a transparent homogenic jelly, which covered completely the small fragment of tissue. It is important that the preparation of the serum agar mixture be made very quickly, to prevent coagulation from taking place before the mixture covers the fragment of tissue. It is also important that the coagulation be complete, so that the presence of small pools of serum between clumps of agar may be prevented.

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The serum and agar were mixed in the ratio of 3 to 1 or 4 to 1, and this proportion gave a fairly good culture medium. Agar dissolved in distilled water was slightly hypotonic. This should have been an advantage, for, according to Carrel and Burrows,[4] plasma diluted with two fifths of its volume of distilled water is a better culture medium than normal plasma.

In this medium I have cultivated the bone marrow of adult guinea pigs. The bone marrow was taken from the femur, was placed in Ringer solution, and was then cut into small pieces the size of a pinhead. Each piece was placed on a cover-glass and covered by the culture medium. After the medium coagulated, the cover-glass was turned over, so as to have the tissue on its under surface. The cover-glass was then placed on a hollow slide, and was incubated at 38° C.

The appearance of living cells in the serum agar medium is the same as that of cells in plasma, and during the first few days there are only very slight differences, quantitatively, in the growth in these two media.

Bone Marrow in Plasma.—As a control on the results with serum agar, the following description of the cultivation of bone marrow in plasma is given: A short time after the incubation of the culture, leucocytes start their emigration from the fragment by active ameboid movements, so that the tissue, after two or three hours, is surrounded by a dense area of living cells. This area rapidly increases in size during the following twenty-four hours, but the increase is less rapid on the second and third days. After three days, the ameboid movements decrease, and after four or five days, movement stops almost completely, the leucocytes become globular, and disintegration begins. It is difficult to keep leucocytes alive for more than five days, but by this time the non-ameboid connective tissue cells of the fragment have started to grow, and have formed an area of new cells around the tissue. The description of the later development of these cells, however, is of no importance for this investigation, and will be omitted.

In serum agar, during the first few days of incubation, the same appearance is shown. The same area of living ameboid cells sur-

[4] *Jour. Exper. Med.*, 1911, xiii, 416.

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rounds the tissue after a few hours, and increases rapidly in size during the first day. But the length of life of the cells is shorter in the serum agar medium than in plasma, and I have usually been unable to keep the leucocytes alive for more than three days, while life for four days was exceptional.

By living cells, I mean leucocytes with active ameboid movements. The term is possibly inadequate, the cells perhaps being alive for some time after they have ceased to move. Ameboid movements are, however, an indisputable sign of life. Moreover, the cessation of movement can be easily determined, while beginning degenerative alterations are not always very evident. As for the life of the leucocytes outside of the organism, there may be some question as to whether we are dealing with real growth and multiplication of cells, or only with an active emigration. It is probable that the latter assumption is the correct one, for there is no evidence of the multiplication of the leucocytes.

The question of whether the leucocytes actually multiply or only emigrate from the bone marrow is of no consequence in our present study, for we are interested not in the multiplication of the leucocytes, but in the growth of the bone marrow. The ameboid movements and the behavior of the leucocytes are regarded as being of great importance, for under the most favorable conditions (growth in plasma), it has been found that the emigration of the leucocytes is always the first stage of the growth of bone marrow. As the growth of the non-ameboid cells of the bone marrow is too slow to make direct measurement practicable, the intensity of the ameboid movements of the leucocytes has been used in these experiments as an index to the influence exerted upon bone marrow by the various culture media.

Guinea pig bone marrow has been cultivated in serum from the same animal (autogenic serum), in that from another guinea pig (homogenic serum), and in that from other mammals (heterogenic serum); *e. g.*, in serum of the rabbit, cat, dog, mouse, goat, and man. In each experiment, three series of cultures were prepared; one in an autogenic, one in a homogenic, and one in a heterogenic medium. In each series, moreover, three or four cultures of bone marrow were made in agar plus unheated normal serum, and three

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or four cultures in agar plus serum which had been previously heated to 56° C. for half an hour. Only absolutely good cultures were used. Cultures were always discarded if there was liquefaction of the medium or an uneven mixture of agar and serum that might give rise to passive movements of leucocytes due to currents in the medium. Moreover, only those experiments were utilized in which all the results in each given series corresponded.

The cultures were examined and the results recorded after six, eighteen, twenty-four, and forty-eight hours. Camera lucida drawings were then prepared, which give a quite exact comparison between the growths in different cultures.

RESULTS.

Even after six hours' incubation, the differences between the emigration of cells in autogenic and homogenic serum were obvious. The area of cells invading the surrounding medium was larger and denser in autogenic than in homogenic serum, a difference which was evident during the two following days (figures 1 and 2).

Autogenic and homogenic serum heated to 56° C. for half an hour proved to be of less value, for in these the area of emigrated cells was smaller than in the corresponding unheated autogenic and homogenic serum media. The difference was most marked between the unheated and the heated autogenic serum, but heat seemed to be a real inactivator for the homogenic serum also. The difference between the growth in unheated and heated sera increased during the first twenty-four hours, and reached a maximum during the second day. At that time, the unheated culture medium was almost totally pervaded by leucocytes. On the third day, however, the growth in the inactivated medium overtook that in the unheated serum mixture.

The length of life of the cells was about the same in autogenic and homogenic sera, whether these were heated or unheated.

The normal heterogenic sera gave rise to a growth of cells which was far less rapid and extensive than that in the autogenic and homogenic sera.

There were, moreover, differences in the growth between different heterogenic sera (figures 2 and 3).

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In normal rabbit serum, after eighteen hours, the piece of bone marrow which was originally red was quite pale, and in the small area of emigrated cells, no ameboid movements were seen. Moreover, after the eighteenth hour, no increase in the area of the emigrated cells was detected.

The growths in the other normal heterogenic sera had the same appearance, but in these death of the cells took place earlier. The sera employed, in the order of the most to the least favorable, are as follows: rabbit, mouse, dog, man, cat, and goat. Goat serum was unsuitable as a culture medium. In goat serum, a very insignificant emigration of leucocytes was seen. After six hours, the leucocytes in goat serum formed a small and irregularly shaped area of cells. Only a few of the leucocytes had ameboid movements, and after a short time, those that showed it ceased altogether to move. No increase of the area of emigration was seen after six hours.

Quite different from the results in autogenic and homogenic sera were those obtained by heating the heterogenic sera, for heating these sera increases their value as culture media. The area of emigrated cells was much larger in heated than in unheated heterogenic serum, and the length of life of the cells that varied from six hours (goat) to eighteen hours (rabbit) in unheated serum, was increased to thirty-six to forty-eight hours in heated heterogenic sera. In heated goat serum, we can see very active ameboid movements after twenty-four hours; and in heated rabbit serum, ameboid movements may be seen even on the third, fourth, and fifth days.

Further experiments similar to those here described are in progress.

In closing, I wish to thank Dr. Carrel, whose technique I have studied, for many valuable suggestions.

CONCLUSIONS.

1. Bone marrow of guinea pigs grows better in autogenic than in homogenic serum.

2. Bone marrow of guinea pigs grows more extensively and rapidly in unheated than in heated autogenic and homogenic serum (56° C. for half an hour).

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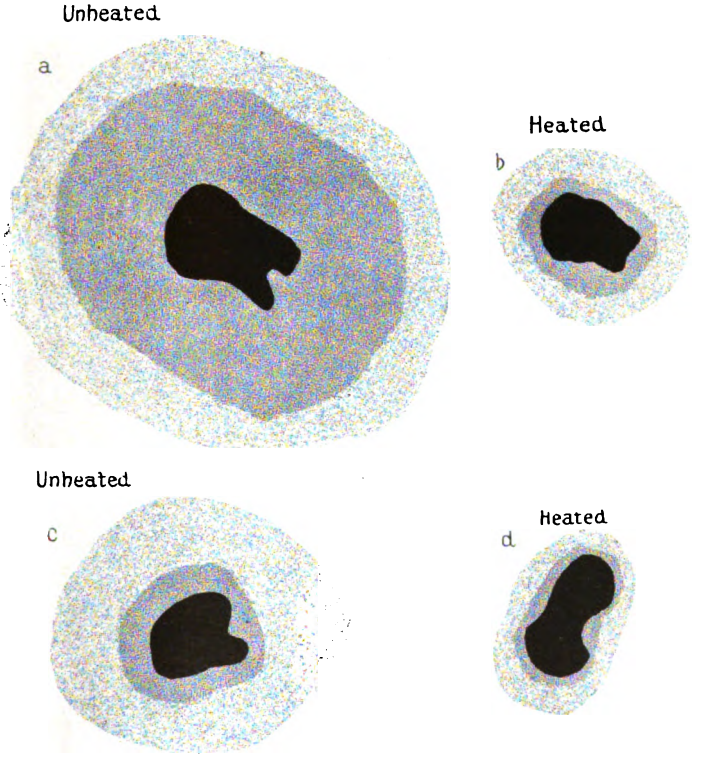


FIG. 1.

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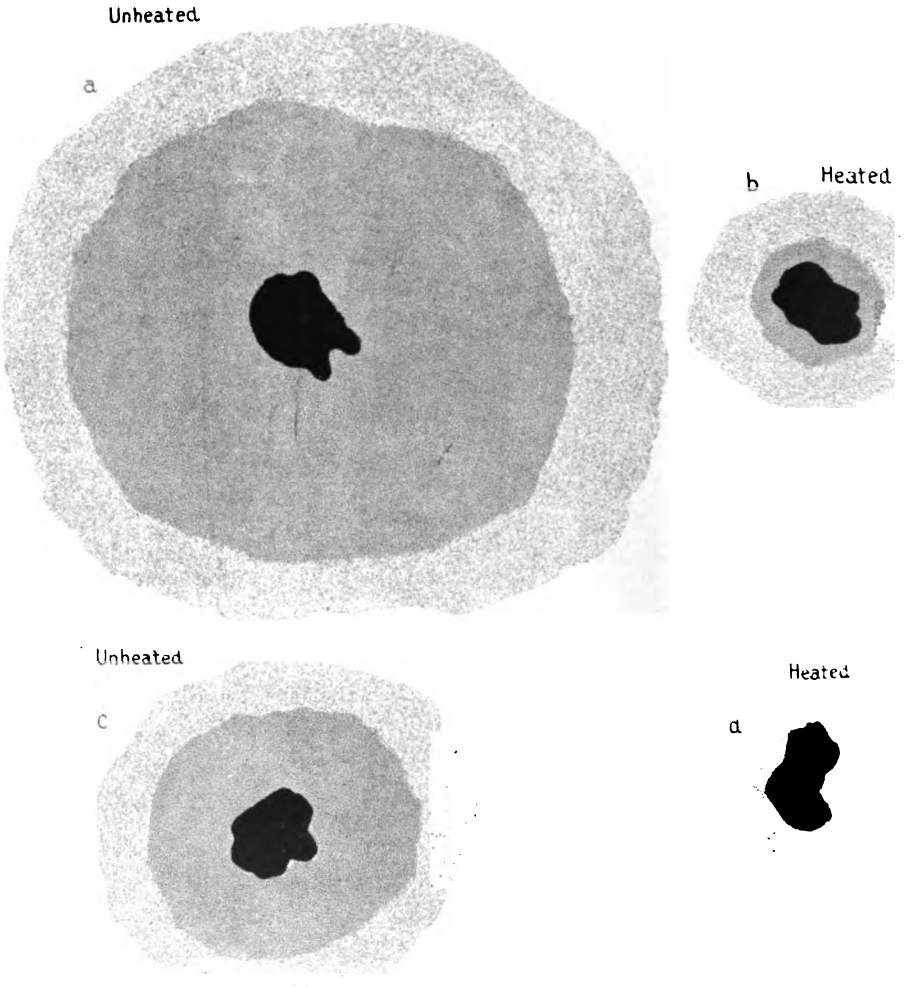


FIG. 2.

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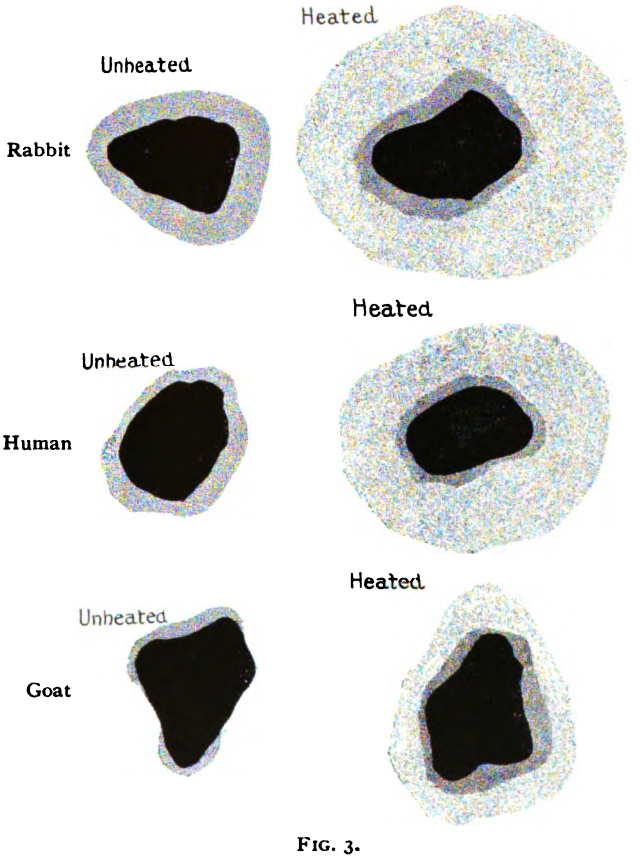


FIG. 3.

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3. In different heterogenic sera, there are differences in the growth of the tissue. In none of the sera examined, was the growth so extensive as in homogenic and autogenic serum.

4. Heated heterogenic serum is a better culture medium and shows more extensive tissue growth than normal unheated heterogenic serum.

EXPLANATION OF PLATES.

Figures 1, 2, and 3 are camera lucida drawings of cultures of guinea pig bone marrow in different sera, heated and unheated. The black spot in the center represents the original piece of tissue, and the surrounding dark grey and light grey areas represent the emigrated leucocytes. The magnification is the same in all the figures.

PLATE 59.

FIG. 1. A six hour old culture of bone marrow in (*a*) unheated autogenic serum, in (*b*) heated autogenic serum, in (*c*) unheated homogenic serum, in (*d*) heated homogenic serum.

PLATE 60.

FIG. 2. A twenty-four hour old culture of bone marrow in (*a*) unheated autogenic serum, in (*b*) heated autogenic serum, in (*c*) unheated homogenic serum, in (*d*) heated homogenic serum.

PLATE 61.

FIG. 3. An eighteen hour old culture of bone marrow in heterogenic sera, heated and unheated.

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April, 1912

CAN THE SPERMATOZOÖN DEVELOP OUTSIDE THE EGG?

JACQUES LOEB AND F. W. BANCROFT

From the Rockefeller Institute, New York

ELEVEN FIGURES

I. INTRODUCTION

The experiments on artificial parthenogenesis have shown that an egg which *naturally* cannot develop without sperm can be caused artificially to develop without being fertilized by sperm. It is natural to raise the question whether a spermatozoön can be caused to develop into a larva without an egg. The experiments on merogony prove that a fragment of the egg deprived of a nucleus can develop into a larva if a spermatozoön enters the egg; this shows that the egg nucleus is not necessary for the development. The phenomena of merogony do not yet prove that the spermatozoön alone can give rise to an embryo. There are several reasons for doubting this possibility. In the first place we have reason to assume that the protoplasm of the egg is the embryo itself. If this be correct we can understand that the spermatozoön might be able to transmit a number of characters to the offspring, without possessing the possibility of becoming an embryo or creating one outside of an egg.

In the second place it is possible that only the protoplasm contains the apparatus necessary for nuclear and cellular division and that a spermatozoön is not able to create this apparatus. In the third place it is possible that the egg protoplasm of each species contains nutritive material and enzymes of so specific a character that it is not likely that we are able to imitate it in the near future. The attempt at causing the spermatozoön to develop without an

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egg is at the same time an attempt to test the validity of the reasons which seem to speak against this possibility.

There is only one paper dealing with this problem, by J. de Meyer.[1] He raised the question whether it is necessary that the spermatozoön should come in contact with the cytoplasm of the egg in order to undergo the first phases of its normal evolution. He used the sperm of Echinus microtuberculatus, which he placed in sea-water containing an extract of the eggs of the same species and found that under these conditions the spermatozoa swelled so as to lose completely their normal appearance. The tail remained unchanged, but the cytoplasmic covering of the head, the middle piece, and the chromatic portion of the head all seemed to swell; and in some cases an indistinct vesicular structure was seen which stained a little stronger than its surroundings, and seemed to be a nucleus. He concludes that incomplete as his results may be, they give a right to conclude, "that the male just as the female cell is capable of evolution under the influence of external agencies" (p. 94).

II. MATERIAL AND METHODS

Our own experiments were carried on on the sperm of the fowl. The sperm was removed aseptically. Only the sperm contained in the lower portion of the vas deferens was used. It was kept in a sterilized moist chamber at about 39° C., but was always used soon after its removal from the animal, not later than three hours after it was taken out. The media used for the culture of the spermatozoön were: egg yolk, egg albumen, chicken blood serum and $\frac{M}{6}$ and $\frac{M}{10}$ Ringer solutions. Slides, cover glasses and instruments were sterilized in a flame and small hanging drops of the various media were inoculated with the spermatozoa. The cover glasses were inverted over hollow slides and sealed with a vaseline and paraffine mixture. In a few cases the eggs were broken into glass vessels and small quantities of sperm injected into the yolk with a capillary pipette. After stated intervals yolk and sperm were taken out for examination with a capillary pipette.

[1] J. de Meyer, Arch. de Biologie, vol. 26, p. 65, 1911.

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III. OBSERVATIONS ON LIVING MATERIAL

When the spermatozoa of the fowl are observed in a hanging drop of white of egg, kept at about 40° C., the first change is seen after fifty or sixty minutes. It consists in the collection of a small amount of some substance having a low refractive index about the middle pieces of some of the spermatozoa. In favorable cases as many as 60 per cent of the spermatozoa may undergo this change. At this time many of these spermatozoa are still swimming. During the course of the next few hours these lowly refractive areas increase in size until they are about half as long as the sperm head and acquire a fairly distinct ellipsoidal outline. Then in many cases the sperm head can be seen to be bent in a horse shoe or spiral shape, and to be included in the wall of the vesicle, which has now become spherical, while the tail of the spermatozoön still remains unchanged or has disappeared without taking any part in the transformation. The next change is an increasing indistinctness in the sperm head, and an increasing refractive power of the whole vesicle so that it can hardly be discriminated at all in the albumen. It is not possible to follow the process farther in unstained material.

In some cases these vesicles instead of being spherical stretch out along the whole side of the sperm head, or may become entirely disconnected from the spermatozoön.

If yolk is used as a culture medium for the sperm essentially the same phenomena occur; and in the various Ringer solutions vesicles containing the sperm heads are also formed, but in the Ringer solution, as a rule, the steps in the formation of these vesicles could not be seen without staining.

IV. OBSERVATIONS ON PRESERVED MATERIAL

When the hanging drops are fixed in Flemming's fluid and stained and examined in Herla's vesuvin and malachite-green mixture, it can be seen that in its early stages the vesicle has distinct walls and a homogeneous unstained fluid of a low refractive index in its interior. This fluid is possibly water and this would

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account for the fact that the vesicle is conspicuous in albumen and yolk, but invisible in Ringer solution.

The vesicle seems to be formed by the imbibition of water by the very thin protoplasmic envelope of the sperm head and middle piece. For after the formation of the vesicle, head, tail and middle piece are, so far as can be seen, unchanged (fig. 1). In many cases the vesicle is seen at the front end of the spermatozoön. Such cases result from the bending of the spermatozoön in the middle piece region, as the series illustrated in figs. 1, 2 and 3 show. The maximum development of the unchanged vesicle is shown in fig. 4.

When the vesicle has reached its full size the material of which its surface is composed seems to wet the sperm head very easily. For in the next stage the sperm head is in contact with the wall of the vesicle along its whole length, and the vesicle has usually assumed a more or less spherical shape (figs. 5 to 8).

Up to this point the transformations were found to take place in the same way in all the media employed, but in the various Ringer solutions the transformation went no farther than this even when the spermatozoa were left in the solutions for forty-eight hours and longer.

In the yolk and albumen, however, the development toward the formation of a nucleus went a little farther. In these media solution of the sperm head took place, which seemed to begin as soon as the head was drawn into the vesicle. For in these media stages like figs. 7, *a*, *c*, *d*, *e*, were quite difficult to find, while in the Ringer solutions such stages, and those in which the sperm head within the vesicle was entirely unchanged were the most frequent transformations observed (figs. 5 and 6).

In preparations that were fixed after the spermatozoa had been in contact with the yolk or albumen only two or three hours the most frequent transformation of the vesicles observed is one in which the head has entirely disappeared while the whole vesicle takes a rather dilute nuclear stain. In a few cases nuclei of this type still show remnants of the sperm head as in fig. 7, *b* and *c*. These appearances would seem to indicate a solution of the chromatin of the sperm head in the contents of the vesicle.

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After the spermatozoa have been left in contact with the culture medium for about eighteen hours no more, or but very few, of these uniformly stained vesicles are to be found. But there are many fairly normal looking nuclei in which the chromatin is all present in the shape of discrete particles resting on the nuclear wall, and in which no linin, or but very small amounts of it, can be seen (figs. 8 to 11). The principal reason for believing that a certain amount of linin is present, even if it is obscured by the chromatin, is that some vesicles are seen, which seem to have broken away from the spermatozoa before they came in contact with the sperm head, and which do not contain any chromatin at all. In these a few strands of linin-like substance may usually be seen traversing the interior of the vesicle; and it is likely that these strands are also present in the other vesicles but cannot be made out on account of the chromatin.

It would seem probable that the chromatin in these nuclei is derived by a condensation of the uniformly distributed chromatin of the previous stage, though it is possible that in a certain number of cases the sperm head breaks up into chromatin particles without a previous complete solution.

Ordinarily no signs of either protoplasm or sperm tails are to be seen in connection with these nuclei but occasionally both may be observed, as in figs. 8*b* and 10*a*; and in several cases it was seen that the middle piece had not been incorporated within the nucleus or vesicle but could be distinctly made out in the tail attached to the vesicle.

When the preparations are fixed in Flemming's fluid, stained with Czaplewsky's carbolic gentian violet, dehydrated and mounted in balsam, clearer pictures of the completed nuclei were obtained (figs. 9 to 11) but the series of intermediate stages in the formation of these nuclei seemed to be entirely different. It was possible to make out a connected series of transformations of the sperm head into nuclei, but since this series contained none of the vesicles so characteristic for the living and glycerine material it must be concluded that this series is composed mainly of artefacts resulting from the shrinkage of the vesicles.

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In these balsam preparations the first change which seemed to take place was a broadening of the sperm head with a decided increase in staining power. Then the sperm head gradually seemed to shorten and assume various irregular and sometimes angular shapes, still retaining its high staining power. Finally these small deeply staining heads seemed to become larger and vesicular, and typical nuclei were formed. Accordingly it would seem that when first formed these vesicles are so delicate that they cannot stand this technique without shrinking all out of shape.

From these experiments we must conclude that in yolk and white of egg the spermatozoön undergoes the transformation into a nucleus. We have not noticed any mitosis or asterformation and we are, therefore, not yet in a position to state that the spermatozoön can undergo mitosis outside the egg.

PLATE 1

EXPLANATION OF FIGURES

All the figures where drawn with a camera from preparations of chicken spermatozoa and nuclei into which the spermatozoa were transformed.

The vesicles in figs. 1 to 6 all stain more lightly than the substance surrounding them, as is represented in fig. 6. To represent the preparations faithfully figs. 1–5 should also have a background similar to that in fig. 6. All except fig. 4 are from preparations fixed in Flemming's fluid.

1 Chicken spermatozoa showing an early stage in the vesicle formation, and the beginning of the bending of the spermatozoön in the middle-piece region. Sperm kept in white of egg two hours, forty-five minutes. Stained and examined in Herla's malachite green and vesuvin mixture. × 1620.

2 Spermatozoa with vesicles as in fig. 1 but more completely bent on themselves in the middle-piece region. All of the loops contain vesicles which are seen exactly from the side in *a* and *c* and slightly from the edge in *b*. From same preparation as fig. 1. × 1620.

3 Spermatozoa similar to those in fig. 2 but seen edge on. From same preparation as figs. 1 and 2. × 1620.

4 Spermatozoön showing the maximum development of the vesicle before the bending of the sperm head begins. Sperm kept in yolk for about four hours. Killed, stained and examined in Herla's. × 640.

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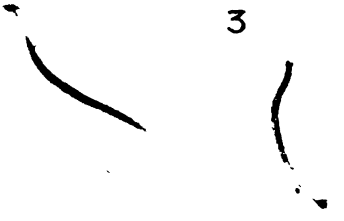
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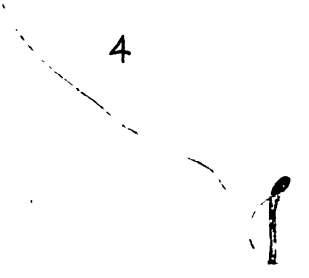
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PLATE 2

EXPLANATION OF FIGURES

5 Sperm heads which have been bent so that they are either within or in contact with the vesicle throughout their entire length. In *b* the flagellum and middle piece of the tail are still visible, but in *a* and *c* these can no longer be made out. Sperm in $\frac{M}{8}$ Ringer solution + 0.35 per cent $\frac{M}{8}$ $NaHCO_3$ for five hours. × 1750.

6 Sperm heads contained in vesicles; *a* still showing remains of tail; *b* shows maximum development that the vesicles attain in Ringer solution. Sperm in $\frac{M}{10}$ Ringer solution four hours. Dark background due to coagulated egg albumen which was mixed with the Ringer solution immediately before fixing to prevent the vesicles being washed off the cover glass Herla's. × 1620.

7 Early stages in the solution and transformation of the sperm heads within the vesicle. From same preparation as figs. 1, 2 and 3.

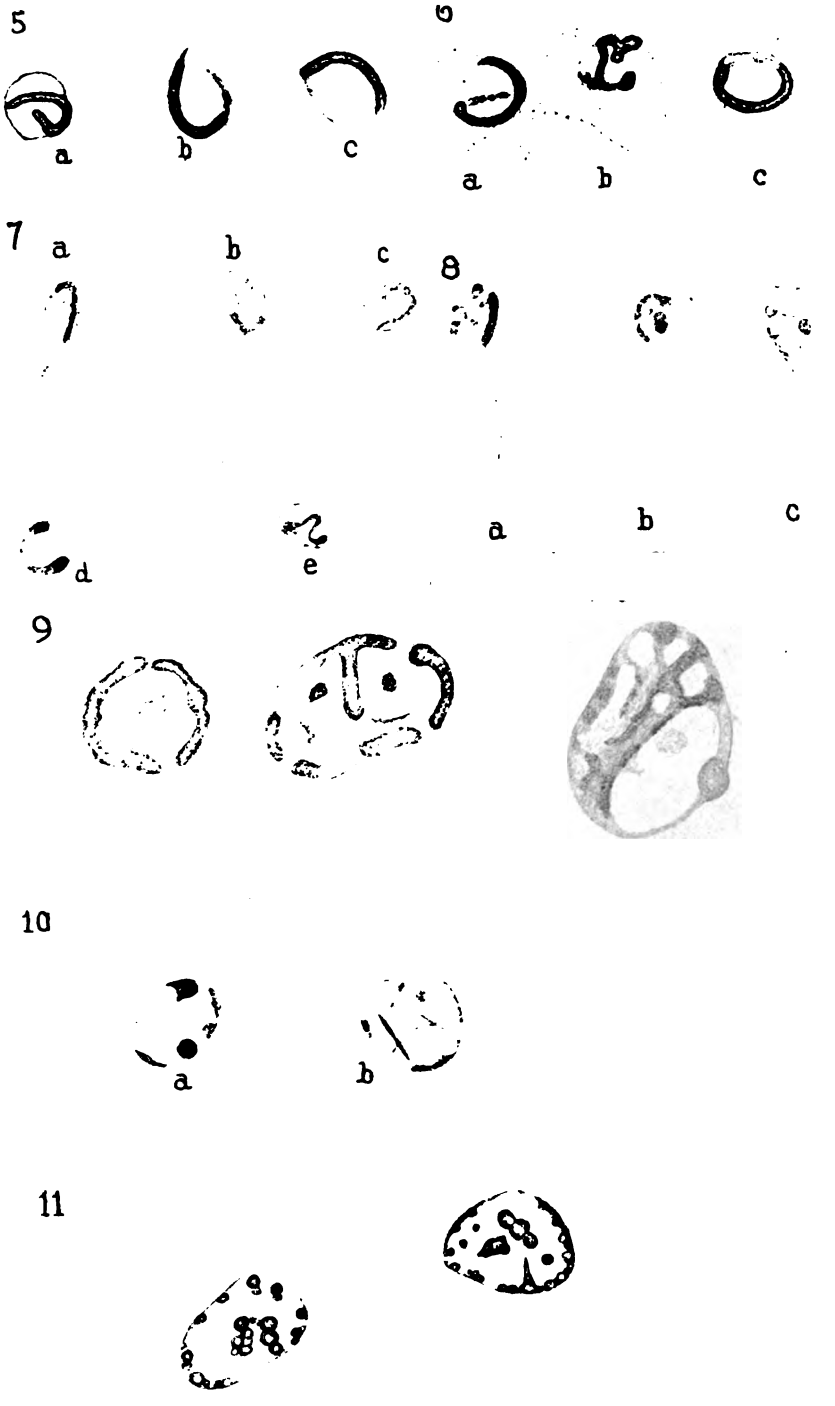
8 Vesicles in which part of the chromatin is present as discrete granules, and part can still be recognized as the portion of the sperm head which adjoins the tail. In *b* two masses of cytoplasm can be seen anteriorly. Herla's. × 1620.

9 Sperm heads transformed into nuclei. Chromatin as far as could be determined all next to the membrane. Sperm in egg albumen twenty-three hours. Gentian violet and balsam. × 5000.

10 Spermatozoa transformed into nuclei. *a* also appears to have some vacuolated protoplasm Sperm in egg albumen twenty-six hours. Herla's. × 3100.

11 Spermatozoa transformed into nuclei. Chromatin all on nuclear membrane. Gentian violet and balsam. × 3100.

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EXPERIMENTAL RESEARCH IN SYPHILIS

WITH ESPECIAL REFERENCE TO SPIROCHÆTA PALLIDA (TREPONEMA PALLIDUM)*

HIDEYO NOGUCHI, M.D.
NEW YORK

According to history, syphilis was not known, or, at least, was not recognized in Europe, until toward the end of the fifteenth century. It is considered probable that it was first introduced into Europe from America by the sailors of Columbus. The epidemic form with which this disease ravaged Europe in those early days has suggested that it is caused by a transmissible virus. No definite search, however, for such an infectious agent was possible until the discovery of the microscope. The first one to describe an organism in syphilitic lesions was Donné, who, in 1837, found a spiral organism to which Müller gave the name of *Vibrio lineola*. As no sharp differentiation between the non-syphilitic and syphilitic lesions had been yet established at that time, the finding of a spiral organism was inadequate to prove that it had any etiologic relation to syphilis. Bassereau, in 1852, rendered a great service by separating definitely the venereal sore from the true chancre, thus giving the basis for accurate investigations.

Research in syphilis became henceforth increasingly active, and the discoveries of the causative organism were announced year after year from different quarters, only to be disproved after a shorter or longer period of refutation and controversy among the investigators at the time. Hallier, in 1869, found in the syphilitic blood his *Coniothecium syphiliticum* and held it as the cause. Lostorfers, in 1872, announced his discovery of minute sparkling granules in the syphilitic blood which was kept for a few days in a moist chamber, but his finding was

* From the Laboratory of The Rockefeller Institute for Medical Research.
* Fenger-Senn Memorial Address, delivered before the Chicago Medical Society, March 13, 1912.

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discredited by Neumann, Biesiadecki, Vajda and others. Then came, in 1878-9, Klebs' discovery in chancre-juice of numerous actively mobile granules and rods which he called *Helicomonades*. In 1882, Birch-Hirschfeld stained in the tissues from gumma, papules and chancres, minute bacteria which others considered as mast-cell granules, while Martineau and Hamonic reported in the same year their alleged success in reproducing the syphilitic lesions in pigs and monkeys with their bouillon cultures of cocci and bacteria. In 1884, the well-known discovery of Lustgarten was heralded from Weigert's laboratory. He demonstrated the presence of a bacillus in certain syphilitic products which resembled the tubercle bacillus discovered by Koch in 1882. The finding was confirmed by a number of reputable bacteriologists, among whom were Doutrelepont, Schütze, Giletti, de Giacomi, Gottstein, Babes and Baumgarten. It soon met with severe criticism, however, by Alvarez, Tavel, Klemperer, Matterstock, Bitter and many others, who not only failed to find this bacillus in the sections of the syphilitic tissues, but found it in other diseases as well as in normal smegma. Thus the discovery of Lustgarten gradually passed from general interest. In spite of Lustgarten's mistake, a great many investigators went further on to find the real organism of syphilis. Most of them described the cocci-like granules in the blood or lesions of syphilitics and claimed to have obtained pure cultures of so-called syphilis bacillus or syphilis coccus from the blood or glands. Van Niessen still asserts that a polymorphous bacillus which he obtained from the syphilitic blood and named *syphilomyces* is the cause of syphilis. He maintains that *Spirochæta pallida* is only one of the life cycles of this bacillus, although Hoffmann points out that there is absolutely no resemblance between the *pallida* and Van Niessen's bacillus in any stage of development. Similar bacilli were reported to be present in the syphilitic blood, by de Lisle and Jullien, Paulsen, Joseph and Piorkowski, but they are now considered to be due to external contamination. There are also certain investigators who claim to have found protozoa in syphilitic products, but their findings were soon disproved as mostly due to the artefact or decomposition products.

THE DISCOVERY OF SPIROCHÆTA PALLIDA

The new era in the experimental research in syphilis began with the successful transmission of syphilitic

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lesions to higher apes by Metchnikoff and Roux in 1903. This opened up the field by furnishing a means of studying the nature of the causative agent of syphilis outside of human subjects. Thus Klingmüller and Baermann determined that the virus of syphilis does not pass through filters and does not, therefore, belong to an ultramicroscopic organism. The finding was confirmed by Metchnikoff. These two findings, the transmissibility to certain animals, and non-filtrability through porcelain bougies of the syphilitic virus, have exerted a great influence in enticing competent microscopists and biologists to attempt anew a thorough search for the virus, for they have learned that the organism lies within the limit of visibility.

It was not, however, until 1905 that an organism which was destined to gain universal recognition as the long-sought virus of syphilis, was viewed for the first time by Fritz Schaudinn in a joint investigation with Erich Hoffmann. Schaudinn found in the aspirated juice from the swollen inguinal glands of a syphilitic a faintly visible, extremely delicate small spirochete in fresh preparations. The organism, stained faintly with Giemsa's solution, was paler than any spirochete known to him at that time; hence he gave it the name of *Spirochæta pallida*,* renaming it *Treponema pallidum* in the same year. Schaudinn and Hoffmann examined a series of syphilitic and non-syphilitic patients and found that the *pallida* was almost always present in syphilitic lesions, but never in other diseases. They have described at the same time another, quite larger, irregularly and less curved spirochete which was found in non-syphilitic as well as syphilitic lesions on genitals. The name of *Spirochæta refringens* was given to this form. The discovery was soon confirmed with amazing rapidity by different investigators who demonstrated the *pallida* in various lesions, blood-vessels, internal organs, blood, spermatozoa, ova, and other body-fluids of syphilitic patients. Through the investigations of Buschke, Fischer, Levaditi, Salmon, Hoffmann, Paschen, Bertarelli, Volpino, Babes, Panea, Flexner, and many others, the *pallida* was demonstrated in abundant number in different organs and tissues of syphilitic children and fetuses.

* For the mere traditional reason I have employed this designation in the present article. When the subject is considered from the systematic or zoologic point of view, the term *Treponema pallidum* is preferred, as has been my custom in other papers.

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The organism was also found by Metchnikoff and Roux in the lesions in monkeys produced directly with human virus or indirectly through transmission of the virus from animal to animal. Soon afterward Truffi, Bertarelli, E. Hoffmann, Uhlenhuth, Mulzer, Nichols and others succeeded in infecting rabbits, guinea-pigs and lower monkeys, and have constantly found the *pallida* in the lesions. The transmissions of the *pallida* from man to monkey, monkey to rabbit, and rabbit to monkey, for many generations, has been carried on by Hoffmann and Nichols. The testicles of rabbits were especially suitable for purifying the *pallida* from the associating organisms, as the latter disappear completely after passing one generation, through the rabbit's testicle. Uhlenhuth and Mulzer were able to produce generalized syphilis in young rabbits by the intracardial inoculation with testicular strains of the *pallida*.

Thus *Spirochæta pallida*, Schaudinn, fulfilled almost all the requirements laid down by Koch before being accepted as the causative agent of syphilis. The only missing link was that a pure culture of this organism should be able to produce the pathologic changes in experimental animals similar to those found in human syphilis.

CULTIVATION OF SPIROCHÆTA PALLIDA

1. *Mixed Cultures.* — Many investigators failed to obtain any growth of the *pallida* outside of the animal body. In 1907, Levaditi, Yamanouchi and others, found that the *pallida* remain motile in a colloidin sac filled with monkey serum and kept for many weeks in the peritoneal cavity of a monkey. The impure cultures thus obtained were non-virulent for any animal. In 1909, Schereschewsky reported that an impure culture of *pallida* may be obtained by inserting a piece of chancre into a high-layer tube of gelatinized horse serum. The success was not uniform, and he says that when the original material contained a virulent strain for rabbits or monkeys no growth in his horse serum was observed. Inversely, an impure growth may take place when the material used was non-virulent. All his cultures were non-pathogenic for monkeys and rabbits. It is difficult to decide whether his impure cultures contained an avirulent *pallida* or a certain spirochete indistinguishable from the *pallida*, because in a culture, morphology alone is no criterion for identifying the *pallida*. In

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1910, Bruckner and Galasesco reported a successful production of syphilitic orchitis in rabbits by means of an impure culture in gelatinized ascitic fluid in which the original syphilitic tissue was still present. Sowade, in 1911, reported a successful generalization of syphilis in a rabbit through the intracardiac inoculation of an impure culture in gelatinized horse serum. In this case the rabbit showed scattered lesions over the body in which the *pallida* were found.

2. *Pure Cultures.*—There are up to the present date three investigators who claim to have succeeded in cultivating *Spirochæta pallida* in pure state, Mühlens, W. H. Hoffmann, and myself. Mühlens published his first article in 1909 and the second in 1910, asserting that he obtained one strain of the *pallida* in pure culture. W. H. Hoffmann, who has assisted Mühlens, continued to work alone a little longer and reported, in 1911, that he was able to isolate five more strains of the same organism as Mühlens. The method of cultivation consisted of the use of Schereschewsky's horse serum for obtaining an impure culture and then purifying it in a horse-serum agar (deep layer). The claim of Mühlens that his spirochete was a *pallida* was based entirely on the similarity of the morphology between the cultivated organism and the *pallida*, because no pathogenicity whatever was possessed by his culture. In his first report, W. H. Hoffmann mentions, also, that none of the strains of the organism cultivated by him was pathogenic. Later, in a brief report, this investigator claims to have produced an orchitis in rabbit by means of one of his cultures. He ascribes his earlier failure to accomplish this to the use of an insufficient quantity of culture. The pathologic changes produced by his culture as described by him were by no means convincing as a syphilitic nature. He succeeded in cultivating back his spirochete which, like all the other cultures of these two workers, developed a penetrating putrefactive odor. In regard to the differentiation of the organism isolated by them, both authors make very little mention, saying that the general characteristics of the culture are indistinguishable from those of *Spirochæta dentium* cultivated by Mühlens in 1906. Like the *dentium*, the spirochete of Mühlens and W. H. Hoffmann grows in a horse-serum agar without the addition of any fresh tissue, and produces a strong putrefactive odor. The differences which their spirochete

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presents in contrast to the *pallida* isolated by me, as will be presently described, are striking. Furthermore, as will be seen later on, there is absolutely no difference between *Treponema microdentium* isolated by me and the so-called *pallida* of these two authors.

Since 1910 I have been working on the cultivation of *Spirochæta pallida* and have succeeded in isolating six different strains from the orchitis material of rabbits and seven directly from chancres, condylomata and skin papules of human subjects. The methods used are different, according to whether the *pallida* is to be cultivated from the orchitis of rabbits or directly from man. For the former, which contains the *pallida* in almost pure state, a fluid medium is preferred. A serum-water containing a piece of sterile fresh tissue is inoculated with the emulsion of the spirochete and cultivated under most strictly anaerobic conditions. After the first generation of the growth is obtained, it is more and more acclimated to the artificial cultural conditions by passing repeated subcultures in the fluid media. Then the *pallida* is transferred to a solid medium containing the suitable nutriments and fresh tissues. If the culture is impure, it can be purified in solid media by a special technic.

On the other hand, a fluid medium is unsuitable for obtaining a growth of the spirochete when the human material is utilized, because the medium undergoes, through the growth of the accompanying bacteria, such changes that it renders the medium unfit for the growth of *pallida*. For this reason, I have resorted to the use of a solid medium consisting of one part of ascitic fluid and two parts of weakly alkaline agar with a piece of sterile fresh tissue at the bottom. The percentage of success depends on the suitableness of the medium which can vary considerably with different specimens of ascitic fluids used, and also on the adaptability on the part of the strains of the *pallida*. The method of purification and other minor technical points will be found in my previous papers.

The six orchitis strains and seven human strains of the *pallida* thus obtained are identical in morphologic and cultural characteristics. They grow slowly and steadily around the tissues and form very faint diffuse undefined colonies extending gradually. The spirochete is strictly anaerobic and requires the presence of a fresh

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sterile tissue for development. It does not attack the protein constituents of the tissue or serum, nor does it produce an odor in growth in any medium. The cultivated *pallida* is less actively motile, but the variety of the movements is characteristic. Under unfavorable cultural conditions its morphology becomes less typical. The growth continues for several weeks. It has been noticed that the *pallida* strains isolated from the rabbit's orchitis grow more luxuriantly in a medium containing rabbit serum, while those from human chancres prefer the ascitic fluid agar. It appears as if the passage of the *pallida* through the rabbit's body modified the biologic property of this organism.

In regard to the pathogenicity, I have succeeded in producing typical orchitis in several rabbits by means of pure cultures of the orchitis strains. With the human strains I was able to produce the initial lesions on the skin of *Macacus rhesus* and *Cercopithecus callitrichus*. The Wassermann reaction developed in these monkeys after the appearance of the induration, several weeks after the inoculation.

The above identification of my cultivated *pallida* strains seems to amply justify my assertion, but I am now in the position to offer further evidences of its identity by means of the immunity and allergic reactions. For a fuller discussion, I will return to this topic later, but a brief statement is made here.

Several series of experiments[1] on rabbits were conducted with the purpose of producing the specific antibodies for the cultivated *pallida* and the tissue *pallida* (rabbit's orchitis). After a prolonged immunization, the serums of these rabbits were tested for the antibodies by means of a specific complement fixation test using the spirochete extract as antigen. It was found that the immune serums prepared with the cultivated *pallida* fix the complement with the antigen derived from the *pallida* of rabbit's orchitis as well as from the culture. The immune serums prepared with the orchitis tissue *pallida* reacted also with both antigens. On the other hand, both sets of the immune serums gave negative reactions with the antigens made of the pure cultures of mouth spirochetes or *Spirochæta refringens*. The mutually interchangeable reaction between the cultivated *pallida* and the tissue *pallida* establishes completely the

1. Partly aided by Dr. J. Bronfenbrenner.

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identity of the two. It is also found that the rabbits sensitized with the orchitis *pallida* by repeated inoculation show the allergic skin reaction to the cultivated *pallida* extract (luetin) as well as to the tissue *pallida* extract. They do not react to the extracts of the *dentium* or *refringens*. This phenomenon adds further evidence that the spirochete cultivated and claimed by me to be the *pallida* is identical with the *pallida* found in the syphilitic tissues.

DIFFERENTIATION OF SPIROCHÆTA PALLIDA AND CERTAIN MORPHOLOGICALLY AND CULTURALLY ALLIED SPECIES

The identification of a microorganism depends on a series of characteristics possessed by each organism. As the deviation of one member from the closely related members of the same family is only gradual and partial, it becomes important to discover as many individual characteristics as possible of each of them. The differentiation may thus become possible by pointing out one or more differences between the two, in spite of the presence of numerous other properties in common. It may happen that two organisms possess almost indistinguishable morphology, but grow differently, while their morpohology may be quite different, yet present similar appearance in growth. Every pathologist knows that the morphologic variations can exist to a considerable extent among different strains of the same organism and offer confusion when one attempts to differentiate it from the morphologic side. Fortunately, we are now in possession of certain indirect methods of identification in such cases, and this often carries more conviction than the most of the other evidences. These indirect methods are the phenomena of immunity and anaphylaxis. While the immunity phenomena are liable to be deprived of their value of identification through occasional group reactions among allied species, yet when it occurs that there is none, it brings identification beyond any dispute. The same is true of the allergy. Another method of identification is applicable only when the organism is pathogenic for certain animals. By the nature of pathogenicity, an organism can be differentiated from the others. But, there is also a difficulty in utilizing this procedure for a definite differentiation, as certain strains of the same pathogenic organism may become attenuated or even avirulent during the cultivation. Hence the

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absence of pathogenicity in this instance constitutes no evidence that the organism is another species.

For the identification of the *pallida* cultivated by me, the above were taken into consideration.

It may be mentioned that I have succeeded in cultivating *Spirochæta macrodentium, Spirochæta microdentium* and *Spirochæta refringens* in pure state and employed them for comparative studies with *Spirochæta pallida.*

Spirochæta pallida is indistinguishable from *Spirochæta microdentium* by the morphologic characteristics, but is well differentiated from the latter by the requirement of tissue for growth, the absence of a putrefactive odor, the pathogenic property, the positive complement fixation with the antiserum produced in animals by immunizing them with pure *pallida* extract (such as the syphilitic orchitis of rabbit), and its capability of inciting an allergic reaction in syphilitic patients. The *macrodentium* and *refringens* can be easily differentiated from the *pallida* by their morphology alone, although they behave quite differently in other respects as well.

Now, turning our attention to the spirochetes cultivated and claimed by Mühlens and W. H. Hoffmann to be the *pallida,* it becomes clear that their spirochete corresponds with *Spirochæta microdentium* in every principal characteristic, and disagrees with the *pallida* obtained by me. It appears quite strange that neither Mühlens nor W. H. Hoffmann has isolated even once a spirochete identical with my strains of the *pallida,* while W. H. Hoffmann states that he obtained his variety, which doubtless belongs to the *microdentium,* from five different lesions. Mühlens, it may be recalled, obtained this variety only once out of nearly eighty different specimens of syphilitic tissues. Whether the *microdentium* is more frequently associated in syphilitic lesions in Germany, it is difficult to say, but so far as my personal experience with numerous chancres and condylomata is concerned, I have never isolated a single strain of the *microdentium* from a syphilitic lesion outside of the oral cavity. It was for the purpose of avoiding such a confusion between the *microdentium* and the *pallida* that I had taken the trouble to use the rabbit's orchitis for cultivation in my first series of work, and later when cultivating the *pallida* directly from human syphilitic tissues, I never utilized the lesions in the mouth.

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TABLE 1.—POINTS OF DIFFERENTIATION OF SPIROCHETES

Varieties of Spirochetes	Requirement for Tissue	Production of Putrefaction Odor	Appearance of Growth	Pathogenicity	Dimension (Av.)	Curves (Average)	Movements	Complement Fixation with the Pallida Immune Serum	Allergic Reaction on Syphilitic Patients
*Spirochæta * pallida*.....	+	—	Diffuse, faint.	+	0.2-0.3 μ x 6-18 μ	Regular, deep. 4-16	Rotation.....	+	+
*Spirochæta * microdentium*.....	—	+	Denser, more discrete.	—	0.2-0.25 μ x 5-18 μ	Regular, fairly deep. 4-20	Rotation.....	—	—
*Spirochæta * macrodentium*.....	+	—	Diffuse, faint.	—	0.25-0.4 μ x 6-18 μ	Less regular. 3-14	Vibratory and rotation.	—	—
*Spirochæta * refringens*.....	—	—	Fairly well defined, faint.	—	0.5-0.75 μ x 6-20 μ	Irregular. 3-8	Wavy and serpentine.	—	—

* They all belong to genus *Treponema*.

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In identifying a cultivated spirochete with *Spirochæta pallida* the following points must be fulfilled:

1. The spirochete must be morphologically correct.
2. It must not produce a putrefactive odor.
3. It requires the addition of fresh tissue for growth.
4. Its extractor emulsion must bind complement with the immune serum (rabbit is preferred) produced by means of repeated injections of the tissue *pallida* (to be obtained from syphilitic orchitis of rabbits).
5. Its extract or emulsion must give an allergic reaction in certain cases of syphilis.
6. It should be pathogenic. The last requirement is highly important, but one cannot exclude the possibility of the organism still being the *pallida,* even if it is avirulent, as long as it fulfils the other five points, because it is not impossible that a strain of *pallida* may become attenuated in cultivation.

MORPHOLOGIC AND PATHOGENIC VARIATIONS IN SPIROCHÆTA PALLIDA

Hitherto but little attention has been paid to certain morphologic and pathogenic variations that exist among different strains of *Spirochæta pallida.* It is true that such variations can hardly be brought out through the usual microscopic examinations of different specimens of syphilitic material. It requires a careful comparative study on a large number of strains either carried through the animal body for many generations, or in pure cultures. I was fortunate in obtaining ten different strains of the *pallida* in the testicles of rabbits and in studying them side by side. During my observations, extending over a period of over one year, I was struck with certain variations in their morphology and pathogenicity. Seven out of ten strains had the typical morphologic features and produced the diffuse orchitis in rabbits within three to four weeks, and progressed for about six to seven weeks. Then the orchitis usually retrogressed. Two strains were somewhat heavier than the average, and produced very hard nodules of cartilaginous consistency within eight weeks. The lesions increased in size very slowly, and remained for many weeks. The lesions on section showed much mucin and were difficult to crush. One strain, derived from a case of malignant syphilis, was somewhat thinner and attained a greater length than the average. It produced a soft diffuse swelling of the testicle within ten to fourteen days, progressing for

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several weeks. These characteristics were maintained unchanged for the entire period of observation.

Among the pure cultures I have observed similar variations in morphology. Of thirteen strains, eight show the typical morphologic features, two thicker and three thinner forms. The morphology of the thinner type resembles that of *Spirochæta microdentium,* and it is almost impossible to differentiate under the microscope. Nevertheless, the other characteristics identify it as the *pallida.* It may be mentioned that different types of the *pallida* can be present together in one lesion, as I was able to obtain a thicker and a thinner form with the average strain.

The above seems to be a highly important distinction, and, if in the study of a still larger number of specimens of the *pallida,* it is maintained, it will throw light on certain important clinical features of the human syphilitic disease.

ALLERGY IN SYPHILIS

The peculiar change of reactivity in the system of individuals infected for some time with certain pathogenic microorganisms, as characterized by a hypersensitiveness to the incorporation of the constituents of the latter in a specific sense, has been recognized for a long time. Thus Koch's tuberculin test, von Pirquet's cutaneous test, Calmette-Wolff-Eisner's ophthalmic test in tuberculosis, the malein test in glanders, and similar reactions in various chronic or acute infectious diseases, have been discovered and become useful aids in diagnosis. Recent studies on anaphylaxis with various proteids, as inaugurated by Theobald Smith and thoroughly investigated by Richet, Otto, Besredka, Rosenau, Anderson, von Pirquet, Shick, Friedberger, Kraus, Dörr, Auer, Lewis, Gay, Southard, Pfeiffer, Wells, and others, clearly established the specific nature of the phenomenon. The most interesting feature of the anaphylactic phenomenon lies in the fact that the hypersensitiveness to a foreign protein develops only when a certain period of cessation of the introduction of the substance is allowed to elapse before the next injection, which then produces the well-known symptoms. The continuation of inoculation at regular short intervals does not confer on the recipient of the foreign substance any anaphylaxis during this period. It is, therefore, more likely to develop an anaphylactic condition in those patients who are infected with

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TABLE 2.—LUETIN REACTION IN VARIOUS SYPHILITIC CONDITIONS AND IN CONTROLS

	Primary Syphilis		Secondary Syphilis				Tertiary Syphilis				Congenital Syphilis				Cerebro-spinal Syphilis		Latent Syphilis		Controls			
	Symptoms Present		Symptoms Present		Symptoms Absent		Symptoms Present		Symptoms Absent		Under 1 Year		Late Cases		Symptoms Present				Normal Individuals		Non-Syphilitic Cases	
Luetin reaction	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
No antisyphilitic treatment.	..	13	..	..	..	..	..	..	..	..	..	..	..	..	..	..	24	6	..	50	..	200
Slight mercurial treatment.	1	12	2	25	..	..	12	..	..	..	5	18	..	..	..	..	..	..	..	..	..	...
Regular mercurial treatment.	..	..	..	..	14	3	31	..	29	1	1	3	15	1	5	5	..	..	..	..	..	...
Salvarsan and mercurial treatment.	..	..	1	..	42	12	..	..	22	3	..	..	9	..	..	..	..	..	..	..	..	...
	1	25	3	25	56	15	43	0	51	4	6	21	24	1	5	5	24	6	0	50	0	200
	26		99				98				52				10		30		250			
	315																					

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certain organisms which remain in their body for a long period, during which their activity undergoes fluctuations either spontaneously, possibly partly owing to the production of certain antagonistic substances by the infected hosts, and partly owing to the nature of the infecting microorganism, or fluctuations through the usual therapeutic interference. The clinical course of syphilis indicates that the infecting agent, *Spirochæta pallida,* fulfils all the requirements that lead to the development of an anaphylactic condition in syphilitic patients. Theoretically one should not expect this condition to appear as long as the activity of the *pallida* is maintained at its maximum, as is the case with the early period of infection. But one can reasonably expect the appearance of this phenomenon when the activity of the organism is abated through the gradually acquired defensive power of the hosts, or under an effective therapeutic control. Thus we may find this condition to be present in later stages of the disease. Likewise, the cases of late hereditary syphilis would behave similarly. It will be seen later that the above theoretical points are well borne out by the practical results to be reported.

Syphilis is a chronic infectious disease, and presents many difficulties in diagnosis. During its very early period, it is principally a disease of dermatologic, genitourinary, and laryngologic fields. There the clinical appearance, demonstration of *Spirochæta pallida* and the Wassermann reaction usually settle the diagnosis. On the other hand, as soon as it enters its chronic course, it manifests most diverse and often obscure symptoms. The direct demonstration of the *pallida* becomes laborious and often impossible, the serum reaction less frequent, and the clinical aspect less decisive. A great many cases of the disease at this period now pass into the fields of medicine, surgery, ophthalmology, neurology, and psychiatry. Here the detection of the allergic condition will doubtless aid in deciding the diagnosis of dubious cases.

Since the discovery of *Spirochæta pallida,* various investigators have attempted to introduce a specific cutaneous reaction based on the allergy in syphilis. Thus, Meirosky, Wolff-Eisner, Munk, Tedeschi, Nobl, Ciuffo, Nicolas-Favre-Gauthier, Neisser-Bruck, Jadassohn and Fontana carried out a series of experiments by means of an extract obtained from syphilitic tissues containing the *pallida.* They were much handicapped

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TABLE 3.—THE

Syphilitic and Non-Syphilitic Serums Used fo
Fixation Tests

Syphilitic Cases	Primary syphilis, untreated
	Primary syphilis, untreated
	Primary syphilis, untreated
	Primary syphilis, untreated
	Primary syphilis, untreated
	Secondary syphilis, slight treatment, general
	Secondary syphilis, slight treatment, general
	Secondary syphilis, moderate treatment, man
	Secondary syphilis, moderate treatment, man
	Secondary syphilis, salvarsan, no symptoms.
	Secondary syphilis, salvarsan, no symptoms.
	Tertiary syphilis, no recent treatment, manif
	Tertiary syphilis, salvarsan and Hg, no symp
	Tertiary syphilis, salvarsan and Hg, sympto
	Tertiary syphilis, Hg treatment, manifest. .
	Tertiary syphilis, Hg treatment, manifest. .
	Late hereditary syphilis, Hg treatment, man
	Late hereditary syphilis, Hg treatment, man
	Late hereditary syphilis, Hg treatment, man
	Late hereditary syphilis, Hg treatment, man
Non-Syphilitic Cases	Gonorrhea
	Tuberculosis
	Chancroid
	Carcinoma
	Dementia præcox
	Leprosy (tuberose type)
	Leprosy (mixed type)
Experimental Syphilis (Rabbits)	Rabbit, syphilitic orchitis, six weeks' duratio
	Rabbit, syphilitic orchitis, six weeks' duratio
	Rabbit, syphilitic orchitis, six weeks' duratio
	Rabbit, syphilitic orchitis, six weeks' duratio
	Rabbit, syphilitic orchitis, six weeks' duratio
	Rabbit, normal
	Rabbit, normal
	Rabbit, normal
	Rabbit, normal
	Rabbit, normal

Explanation: + = positive; ½ + = weak

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by not having a pure *pallida* extract for such purposes. One can imagine the way in which an extract containing various bacteria besides the *pallida* would react. With such an impure antigen, some of them obtained quite favorable results, while others were unable to come to any conclusive result.

After obtaining the pure cultures of several strains of the *pallida,* in 1910-11, I commenced my experimental work on rabbits with the purpose of ascertaining if these animals could not be made allergic to the extract of pure *pallida.* By repeated intravenous injections of the *pallida* antigen into the rabbits for several months and then giving them a month's rest, I tested them with the extract, which was termed "luetin," given intradermally. A proper control was provided. They all reacted to the luetin with marked inflammation, some leading to pustulation in several days. No normal rabbit reacted. While I was still working with the animals, Professor Welch suggested that I make the test on human subjects. Through his encouragement, I commenced the work at once at different dispensaries and hospitals with the cooperation of the physicians in charge.

My series comprised several hundred cases, including syphilis, parasyphilis, non-syphilitic diseases, and normal individuals.

In the series just referred to, the luetin was made from only two strains of pure cultures of the *pallida.* Since then, I have been preparing it with at least six different strains, thus securing a polyvalent antigen. The luetin has been recently distributed to certain hospitals in this country and Europe and their results are not reported as yet. The first report on this subject published is by Cohen, who is applying the test to the ophthalmologic conditions. His results show that the reaction is specific and offers much aid in diagnosis where either the seroreaction or the clinical symptoms are indecisive. The second report is by Orleman-Robinson, who applied the reaction to dermatologic conditions at several skin clinics in New York. The results of this investigator also confirm the specificity. The reaction was positive in all tertiary and hereditary cases, and absent in the primary and untreated secondary syphilis. It was not present in a large number of skin cases, including psoriasis, epithelioma, acne vulgaris, erythema multiformis, urticaria, alopecia areata, trichophytosis,

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erythema toxicum, bromid eruption, sycosis, scabies, pityriasis rosea, tinea versicolor, eczema, ulcus cruris, Darier's disease, eczema seborrheicum, and also pulmonary tuberculosis. Both investigators did their work at their clinics under the joint observations of their colleagues.

In certain cases of tertiary and hereditary syphilis, they have observed the so-called *Umstimmung* of Neisser, in which the control injection also reacted more or less markedly. This confirms my earlier observations.

Regarding the varieties of the luetin reaction, I have made the following types:

DESCRIPTION OF THE REACTIONS

Normal or Negative Reactions.—After applying the emulsions, both luetin and control, to about fifty normal individuals, I was able to determine the variations and limitations of the reactions that follow intradermic administration in the normal skin of a man. In the majority of normal persons, there appears, after twenty-four hours, a small erythematous area at and around the point of injection. No pain or itching sensation is experienced. This reaction gradually recedes within forty-eight hours and leaves no induration. In certain individuals, the reaction may reach a stage of small papule formation after twenty-four to forty-eight hours, after which and within seventy-two hours it commences to subside. No induration is left behind, although occasionally slight yellowish pigmentation may result from mild ecchymosis.

Positive Reactions. — According to the manner and intensity with which the skin of syphilitics responds to the introduction of luetin, one may distinguish the following varieties of effects:

A. Papular Form: A large, raised, reddish, indurated papule, usually from 5 to 10 mm. in diameter, makes its appearance in twenty-four to forty-eight hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degree of induration slowly increase during the following three or four days, after which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish-red. The induration disappears within one week, except in certain instances in which a trace of the reaction may persist for a longer period. This latter effect is usually seen

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among patients with secondary syphilis under regular mercurial treatment in whom there are no manifest lesions at the time of making the skin test. Patients with congenital syphilis also show this reaction in early period of life.

B. Pustular Form: The beginning and course of this reaction resemble the papular form until about the fourth day, when the inflammatory processes commence to progress. The surface of the indurated, round papule becomes mildly edematous, and multiple miliary vesicles occasionally form. At the same time, a beginning central softening of the papule can be seen. Within the next twenty-four hours, the papule changes into a vesicle filled at first with a semi-opaque serum that later becomes definitely purulent. Soon after this, the pustule ruptures spontaneously or after slight friction or pressure. The margin of the broken pustule remains indurated, while the defect caused by the escape of the pustular content becomes quickly covered by a crust that falls off within a few days. About this time the induration usually disappears, leaving almost no scar after healing. There is a wide range of variation in the degree of intensity of the reaction described in different cases, as some show rather small pustules, while in others the pustule is much larger. This reaction was found almost constantly in patients with tertiary or late hereditary syphilis.

C. Torpid Form: In rare instances, the injection-sites fade away to almost invisible points within three or four days, so that they may be passed over as negative reactions. But sometimes these spots suddenly light up again after ten days, or even longer, and progress to small pustular formation. The course of this pustule is similar to that described for the preceding form.

This form of reaction has been observed in a case of primary syphilis, in one of hereditary syphilis, and in two cases of secondary syphilis, all being under mercurial treatment.

Neither in syphilitics nor in parasyphilitics did a marked constitutional effect follow the intradermic inoculation of the luetin. In most positive cases, a slight rise in temperature took place lasting for one day. In three tertiary cases and in one hereditary case, however, general malaise, loss of appetite and diarrhea were noted.

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The final estimation of the luetin test awaits future investigations by a large number of observers. In the meanwhile, I consider it fairly accurate to state that in this reaction one has a specific test for syphilis. Its more constant presence in the late stage of syphilis than the serum reaction may be of special advantage to those who have to deal with this class of cases.

From my limited observations, it appears that the allergic condition of skin in syphilitic patients persists as long as the infecting agent still survives somewhere in the body, and it requires a most energetic treatment to remove it. Should the destruction of the *pallida* be complete, the allergy must also cease to exist beyond a certain length of time. In animal experiments the luetin reaction no longer appeared after a period of several months. In several instances of human syphilis, in which the symptoms and serum reaction had disappeared under the treatment with salvarsan, the subjects failed to respond to luetin reaction after from eight to twelve months, and the patients still remain in excellent health. It will be of great importance if the luetin reaction can be employed for determining a cure. I have, however, seen cases in which the disease persists in spite of the treatment, and the patients do not give a positive luetin reaction. This class of cases shows undoubtedly an unfavorable prognosis.

SPIROCHÆTA PALLIDA AND THE WASSERMANN REACTION

Although the discovery of the Wassermann reaction was due to the assumption that a syphilitic serum containing the specific antibodies fixes the complement when mixed with an extract containing *Spirochæta pallida,* the real cause of this interesting phenomenon is now generally known not to be of the nature of a specific complement fixation brought about through the combination of the syphilitic antigen (*Spirochæta pallida*) and antibodies, in the strict sense of the term. The discovery of Landsteiner, Müller and Pötzl and Porges and Meier, that an alcoholic extract of syphilitic as well as normal tissues yields practically the same results as an aqueous extract of a syphilitic organ, originally recommended by Wassermann and Bruck, and the fact that the Wassermann reaction occurs also in non-syphilitic diseases (leprosy, frambesia, malaria, etc.) as observed and confirmed by later investigators, made the original

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antigen-antibody view untenable. Further, the extensive series of experiments of myself, later with Bronfenbrenner, conclusively proved that the active principles of the so-called antigens in the Wassermann reaction are present in the lipoidal substances of the tissues, irrespective of whether they are derived from syphilitic or non-syphilitic human subjects or animals.

In spite of these facts, certain investigators still adhere to the original view without, however, any definite evidence in favor of such an assumption. Since the causative organism, *Spirochæta pallida,* has now been cultivated in pure state and identified through its pathogenicity and other biologic properties, such as the capability of inciting the Wassermann reaction in experimental animals, it is now possible to find out by exact experiments to what extent *Spirochæta pallida* plays the rôle of the so-called antigen in the Wassermann reaction.

In order to settle the above question, several series of experiments were carried out on repeated occasions in regard to the following points:

1. Can syphilitic serums giving the positive Wassermann reaction by means of the lipoidal "antigen" also bind complement when the lipoids are replaced by the extract or emulsion of *Spirochæta pallida?*

2. Is it possible to intensify the ordinary Wassermann reaction in syphilitic serums by adding the extract or emulsion of the *pallida?*

The second problem was set up through the supposition that certain syphilitic serums giving weak or negative reactions with the lipoidal "antigen," may nevertheless contain sufficient antibodies which, while on account of the absence or insufficiency of the *pallida* substance are only partially detected or remain unrevealed, may be brought into evidence by the addition of the real antigen. This assumption originates from the conception of Citron, who considers that the lipoidal "antigen" is merely an intermediator to bring about the combination of the antibody and real antigen in the syphilitic serums where they are supposed to be existing side by side without entering combination until the lipoids are added.

In deciding the first point, I have prepared the aqueous extract and emulsion of *Spirochæta pallida* derived from two different modes of cultivation, namely, one in the testicles of rabbits and the other in artificial

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culture media. Both materials were pure and contained an enormous quantity of *Spirochæta pallida.* In making up the extract I have employed several strains of the *pallida* in order to obtain a polyvalent antigen. In case of syphilitic orchitis of rabbits, the indurated testicles of each strain were removed from the animals (under usual aseptic precaution), and these different strains were put together in a sealable porcelain jar for grinding by means of marbles in a shaking-machine. An adequate quantity of sterile physiologic salt solution was added before the grinding. The disintegration of the *pallida* was almost complete after six hours. The emulsion thus obtained was then carefully transferred to a sterile bottle, heated to 60 C for thirty minutes and 0.4 per cent. phenol (carbolic acid) was added. This was used as the antigen. For the control a similar extract with normal rabbit's testicles was prepared.

For making the antigen from pure cultures of the *pallida,* the technic used was identical with the foregoing. The control extract was prepared with uninoculated media. The culture medium consisted of one part of ascitic fluid and two parts of a weakly alkaline agar to which a piece of fresh sterile rabbit's kidney was added. For the antigen only the colonies of the *pallida* were employed, the tissue (kidney) being previously removed.

After determining the anticomplementary titer of each emulsion, I have tested a large number of syphilitic serums from human subjects and also from rabbits with syphilitic orchitis. Non-syphilitic serums both of man and rabbits were used as controls. Throughout the entire series of experiments, the antihuman hemolytic system (Noguchi) was used. The serums were inactivated at 55 C. for thirty minutes. This precaution is absolutely necessary as the antigens contain various proteids capable of causing a proteotropic fixation with unheated serums.

In order to know the relation quantitatively between the Wassermann reaction produced by the lipoidal "antigen" and the fixation by the *pallida* extract as antigen, I have titrated the positive serums for their fixing-power simultaneously with the lipoidal and *pallida* antigens.

The Wassermann reaction is present in most of the syphilitic cases here studied and also in two cases of tuberous and mixed type of leprosy. It is more pronounced in untreated early cases and in hereditary cases

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than in the treated cases. It is present in tertiary cases in a lower percentage. On the other hand, a positive reaction was obtained with the *pallida* extracts in certain cases with weak or negative Wassermann reactions. These occurred, however, only when the patients were under treatment or had been syphilized many years ago without being cured. It is also remarkable to notice that in many cases of tertiary syphilis and later hereditary cases, there was a partial fixation, irrespective of the absence or presence of fixation as indicated by the lipoidal antigen. No fixation was obtained with the *pallida* antigen made from pure culture in the case of leprosy, while the extract from syphilitic as well as normal rabbit's testicles gave a positive reaction, due, doubtless, to the lipoidal content of the latter.

These facts may be considered as deciding (1) that the Wassermann reaction is caused by the lipotropic substances, but not by the antibodies which combine specifically with the *pallida* antigen; (2) that the fixation produced by the culture *pallida* antigen with certain syphilitic serums is caused by the specific antibodies contained in the latter and may constitute a specific diagnostic method for syphilis; (3) that the fixation caused by the testicular extracts behaves like the culture *pallida* extract in the majority of cases, but when the serums (syphilitic or leprous) contain abundant lipotropic substances, it may give a Wassermann reaction as well, which is not the case with the culture *pallida* antigen; and finally (4), that in the serum of rabbits with active syphilitic orchitis there is no indication of the presence of a sufficient amount of the antibodies for the *pallida* antigen, although it gives a strong Wassermann reaction. It remains to be seen when and under what conditions the specific antibodies for the *pallida* will most abundantly be formed in syphilitic patients. At all events it is rather remarkable that the amount of the antibodies detectable by the *pallida* antigen in these cases was so small as compared with certain other infectious diseases, in this respect. It is not improbable that those who come under our care belong to a class of individuals with comparatively less resistance to the *pallida* and are incapable of producing sufficient antibodies, while there are many who respond to the infection with more vigorous formation of the antibodies and reduce the infection to a harmless latency or even destroy the *pallida* completely. This latter class of infected persons

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do not, of course, frequent our clinics. If this is the case, it would be of immense prognostic importance to check a patient from the beginning of infection by the complement fixation test with the *pallida* antigen, thereby determining the resistance of the patient against the disease.

We have in the Wassermann reaction a fair measure of activity of the infecting agent, and now we will have in the *pallida* fixation reaction a gauge for the defensive activity of the infected host.

DEMONSTRATIONS

In order to illustrate the foregoing address with practical demonstrations, I have exhibited the macroscopic and microscopic (dark field) specimens of *Spirochæta pallida, microdentium, macrodentium* and *refringens* in pure cultures. For the assistance and necessary arrangements for the occasion, I express sincere thanks to Professor Zeit and his assistants.

For the demonstration of the luetin reaction, I am under deep obligation to Dr. W. L. Baum, Dr. B. C. Corbus, Dr. O. Stein and Dr. J. Grinker, who placed a series of syphilitic, parasyphilitic and non-syphilitic cases at my disposal. For the privilege of using the clinic of the Chicago Post-Graduate Medical School during the preparation for the demonstration, my thanks are due to its president, Dr. Emil Ries. It is my pleasant duty to express my gratitude to Dr. Corbus, who has also given me a series of syphilitic cases (private), for which he has been keeping all important data for a number of years. The most important feature of his private cases lies in the fact that the patients were treated by him according to a biologic measure, namely, in regard to the Wassermann reaction. The patients selected by him were those who had been or are still being treated with salvarsan and mercury, and had lost the Wassermann reaction a long or short time ago. As will be seen in the following records, some of the cases with negative Wassermann showed a positive luetin reaction, while others were negative. It seems to be highly important to keep track of the latter group of cases for many years to come, since, in my opinion, these patients with negative, clinical, serologic and allergic findings may belong among the cured patients. This assumption may not, however, apply to those patients who had been treated energetically before the allergic state of the skin was developed (such as cases of primary syphilis), because in these instances the luetin reaction may never be manifest. The prognostic value of the luetin reaction would, therefore, become obvious only in those who had had a generalized syphilis before the treatment.

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Below I present brief records of the cases used for the demonstration. The Wassermann reaction in these cases was made by Dr. Corbus.

CASE 1.—N. M., American woman, aged 29, clinic patient of Drs. Baum and Corbus. Present condition: Early secondary lues. Treatment: Mercury rubbings. Wassermann reaction not taken. Luetin mild positive.

CASE 2.—F. B., white man, aged 44, clinic patient of Drs. Baum and Corbus. Present condition: Latent secondaries. Chancre in October, 1910. Treatment: Mercury rubbings and internal. Wassermann positive. Luetin papular distinct (positive).

CASE 3.—J. D., American man, aged 34, clinic patient of Drs. Baum and Corbus. Present condition: Latent secondaries. Infected two years ago. Treatment: Mercury rubbings. Salvarsan injected in oil suspension 0.6 gm. Wassermann positive. Luetin mild positive.

CASE 4.—S. B., American man, aged 26, clinic patient of Drs. Baum and Corbus. Present condition: Recurrent secondaries. Treatment: Mercury rubbings intermittent. Wassermann positive. Luetin distinct papular (positive).

CASE 5.—W. H. W., American man, aged 23, clinic patient of Drs. Baum and Corbus. Present condition: Marked secondaries. Treatment: Salvarsan in oil suspension, February, 1912. Mercury intermittent. Wassermann not made. Luetin distinct papular (positive).

CASE 6.—L. J., colored man, aged 23, clinic patient of Drs. Baum and Corbus. Present condition: Secondaries. Eighteen months since treatment. Treatment: Intravenous injection of salvarsan eight months ago. Mercury rubbings. Wassermann positive. Luetin suppuration on both arms. No difference between control and luetin (*Umstimmung?*).

CASE 7.—C. K., German man, aged 22, clinic patient of Drs. Baum and Corbus. Present condition: Secondary period. No symptoms. Chancre four years ago. Treatment: Mercury rubbings. Salvarsan—intravenous injection May 20, 1911; June 7, 1911; October, 1911; November, 1911. Wassermann not taken. Luetin papular reaction distinct (positive).

CASE 8.—H. K., American man, aged 46, clinic patient of Drs. Baum and Corbus. Present condition: Cerebral gumma. Treatment: Mercury intermittent. Wassermann positive. Luetin large full indurated papule. Typical positive reaction.

CASE 9.—J. S., colored man, aged 34, clinic patient of Drs. Baum and Corbus. Present condition: Probable tertiary. No history. Treatment: Internal about one year. Wassermann positive. Luetin secondary infection. All reactions, both control and luetin.

CASE 10.—A. M., American woman, aged 21, clinic patient of Dr. Stein. Present condition: Congenital lues. Treatment:

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None at any time. Wassermann negative. Luetin distinct papular (positive).

CASE 11.—C. H., American man, aged 16, clinic patient of Drs. Baum and Corbus. Present condition: Congenital lues. Treatment: Mercury intermittent. Wassermann positive. Luetin pustular reaction (positive). Slight *Umstimmung*.

CASE 12.—W. J. H., American man, aged 42, clinic patient of Dr. Grinker. Present condition: Paresis. Probably infected twenty years ago. Treatment: Neglected. Salvarsan injected intravenously by Dr. Corbus Dec. 1, 1911, and Feb. 5, 1912. Vigorous mercury rubbings between salvarsan injections. Wassermann positive, Nov. 28, 1911. Luetin distinct papular (positive).

CASE 13.—Control case. S. C., colored man, aged 37, clinic patient of Drs. Baum and Corbus. Present condition: No history of initial lesion. No secondaries. Believed to be infected twenty years ago. Treatment: Mercury intermittent. Wassermann negative. Luetin negative.

CASE 14.—C. D., American man, aged 26, private patient of Dr. Corbus, treated after the biologic method. Present condition: Probably cured. Tonsillar chancre, November, 1910. Treatment: Salvarsan injected intramuscularly, Nov. 7, 1910, and Dec. 15, 1910. Wassermann positive, Nov. 28, 1910; negative, Jan. 23, 1911; negative, May 9, 1911; negative, March 12, 1912. Luetin negative.

CASE 15.—F. S., American man, aged 22, private patient of Dr. Corbus, treated after the biologic method. Present condition: Early secondaries. Last symptoms, Sept. 4, 1911. Treatment: Injected salvarsan in oil suspension, Sept. 4, 1911; intravenously, Feb. 6, 1912. Vigorous mercury rubbings between salvarsan injections. Wassermann negative, Jan. 16, 1912. Luetin distinct papular (positive).

CASE 16.—C. L., American man, aged 38, private patient of Dr. Corbus, treated after the biologic method. Present condition: Incipient tabes. Infected eleven years ago. Treatment: Salvarsan injected intramuscularly, Jan. 9, 1911, and March 5, 1911; intravenously, Oct. 6, 1911, and Jan. 13, 1912. Vigorous mercury rubbings between injections. Wassermann positive, Nov. 28, 1910; positive, Feb. 23, 1911; negative, July 7, 1911; negative, Oct. 6, 1911; negative, Jan. 9, 1912. Luetin negative.

CASE 17.—M. A., American man, aged 36, private patient of Dr. Corbus, treated after the biologic method. Present condition: Secondaries. Last appearance of palmar syphilid, seven months ago. Treatment: Injected salvarsan intramuscularly, Jan. 28, 1911; intravenously, March 18, 1911; intramuscularly, August, 1911. Vigorous mercury rubbings between salvarsan injections. Wassermann negative, Aug. 22, 1911; negative, Jan. 16, 1912. Luetin distinct papular (positive).

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CASE 18.—P. S., American man, aged 22, private patient of Dr. Corbus, treated after the biologic method. Present condition: Cured. Primary lesion, Jan. 21, 1909. Treatment: Vigorous mercury rubbings. Cure obtained before salvarsan was administered. Injected salvarsan intramuscularly, Jan. 17, 1911. Wassermann negative, Dec. 10, 1910; Feb. 15, 1911; April 15, 1911; Aug. 1, 1911, and Dec. 15, 1911. Luetin negative.

CASE 19.—O. J. G., American man, aged 31, private patient of Dr. Corbus, treated after the biologic method. Present condition: Latent secondaries. Chancre, September, 1905. Last symptoms, Oct. 20, 1910. Treatment: Neglected at first. Injected salvarsan intramuscularly, Oct. 20, 1910, and Dec. 11, 1910; intravenously, Sept. 2, 1911, and March 9, 1912. Vigorous mercury rubbings between salvarsan injections. Wassermann positive, Nov. 11, 1910; negative, Jan. 1, 1910; positive, March 8, 1912. Luetin distinct papular (positive).

CASE 20.—A. G., American man, aged 20, private patient of Dr. Corbus, treated after the biologic method. Present condition: Secondaries. Infected Jan. 1, 1911. Last symptoms May 6, 1911. Treatment: Internal. Salvarsan injected in oil suspension, 0.6 gm., May 6, 1911; intravenously, 0.4 gm., Feb. 24, 1911. Vigorous mercury rubbings between injections of salvarsan. Wassermann positive, April 20, 1911, and March 4, 1912. Luetin distinct papular (positive).

Two patients with acne vulgaris, both adults, were also tested with the luetin and gave a negative reaction.

BIBLIOGRAPHY

Bertarelli and Volpino: Weitere Untersuchungen über die Gegenwart der *Spirochæta pallida* in den Schnitten primärer, sekundärer und tertiärer Syphilis, Centralbl. f. Bakteriol., 1906, xli, 74.

Brückner and Galasesco: Orchite syphilitique chez la lapin par cultures impures de spirochètes, Compt. rend. Soc. de biol., 1910, lxviii, 684.

Buschke and Fischer: Über das Vorkommen von Spirochäten in inneren Organen eines syphilitischen Kindes, Deutsch. med. Wchnschr., 1905, No. 20, p. 791.

Cohen, Martin: Noguchi's Cutaneous Luetin Reaction and Its Application in Ophthalmology, Arch. of Ophthal., 1912, xli, 8.

Flexner: *Spirochæta (Treponema) pallida* and Syphilis, Jour. Exper. Med., 1907, ix, 464.

Fränkel, C.: Über das Vorkommen der *Spirochæta pallida* bei Syphilis, München. med. Wchnschr., 1905, No. 24, p. 1129; über einen Fall von angeborener Dünndarmsyphilis nebst Bemerkungen über die ätiologische Bedeutung der *Spirochæta pallida*, München. med. Wchnschr., 1907, No. 32, p. 1576.

Hoffmann, Erich: Die Ätiologie der Syphilis nach dem gegenwärtigen Stand unserer Kenntnisse, Verhandl. d. deutsch. derm. Gesellsch., IX Kongress, Berlin, 1907, Part I, p. 115; Atlas der ätiologischen und experimenteller Syphilisforschungen, Berlin, 1908, J. Springer; Berichte über neuere Versuche die *Spirochæta pallida* rein zu züchten und auf Tiere zu übertragen, Sitzungsber. Niederrhein. Gesellsch. f. Naturf. und Heilk. in Bonn, Oct. 23, 1911; Ätiologie der Syphilis, in Handbuch der Geschlechtskrankh., 1912, Wien.

Hoffmann, W. H.: Reinzüchtung der *Spirochæta pallida*, Ztschr. f. Hyg., 1911, lxviii, 27; Die Übertragung der Syphilis auf Kaninchen mittels reingezüchteter Spirochäten vom Menschen, Deutsch. med.

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Wchnschr., 1911, No. 34, p. 1546; Die Reinzüchtung der *Spirochæta pallida*, Berl. klin. Wchnschr., 1911, No. 48, p. 2160.

Levaditi: A propos de l'impregnation au nitrite d'argent des spirochètes sur coupes, Compt. rend. Soc. de biol., 1906, lviii, 67; L'histologie pathologique de la syphilis hérédit. dans ses rapports avec la *Spirochæta pallida*, Ann. de l'Inst. Pasteur, xxv, No. 1, p. 41.

Metchnikoff and Roux: Études expérimentales sur la syphilis, Ann. de l'Inst. Pasteur, 1903, 1904, 1905, 1906, 1907; Recherches microbiologiques sur la syphilis, Bull. de l'Acad. de méd., Paris, lxix, No. 20, and Bull. méd., 1905, p. 441.

Mühlens: Reinzüchtung einer Spirochäte (*Spirochæta pallida?*) aus einer syphilitischen Drüse, Deutsch. med. Wchnschr., 1909, xxxv, 1261; Über Züchtungsversuche der *Spirochæta pallida* und *Spirochæta refringens* sowie Tierversuche mit den kultivierten Spirochäten, Klin. Jahrb., 1910, xxiii, 339.

Noguchi: Pure Cultivation of Pathogenic *Treponema pallidum*, THE JOURNAL A. M. A., July 8, 1911, p. 102; Über die Gewinnung der Reinkulturen von pathogenen *Spirochæta pallida* und *Spirochæta pertenuis*, München. med. Wchnschr., 1901, No. 29, p. 1550; A Method for Pure Cultivation of Pathogenic *Treponema pallidum*, Jour. Exper. Med., 1911, xiv, 99; Direct Cultivation of *Treponema pallidum* Pathogenic for Monkeys, Jour. Exper. Med., 1912, xv, 90; Cultural Studies on Mouth Spirochetes, Jour. Exper. Med., 1912, xv, 81; A Cutaneous Reaction in Syphilis, Jour. Exper. Med., 1911, xv, 557; Hautallergie bei Syphilis; ihre diagnostische und prognostische Bedeutung, München. med. Wchnschr., 1911, No. 45, p. 2372.

Schaudinn and Hoffmann, Erich: Vorl. Bericht über das Vorkommen von *Spirochæta pallida* in syphilitischen Krankheitsprodukten und bei Papillomen, Arb. a. d. k. Gsndhtsamte., 1905. xxii, 527; Über Spirochätenbefunde in Lymphdrüsensaft Syphilitiker, Deutsch. med. Wchnschr., 1905, No. 18, p. 711; Über *Spirochæta pallida* bei Syphilis und die Unterschiede dieser Form gegenüber anderen Arten dieser Gattung, Berl. klin. Wchnschr., 1905, No. 22, p. 673.

Schereschewsky: Züchtung der *Spirochæta pallida* (Schaudinn), Wchnschr., 1905, pp. 1665, 1728.

Scherschewsky: Züchtung der *Spirochæta pallida* (Schaudinn), Deutsch. med. Wchnschr., 1909, xxxv, 825; Weitere Mittheil. über die Züchtung der *Spirochæta pallida*, Deutsch. med. Wchnschr., 1909, xxxv, 1260; Bisherige Erfahrungen mit der gezüchteten *Spirochæta pallida*, Deutsch. med. Wchnschr., 1909, xxxv, 1652; Erkennung des Syphiliserregers auf dem Wege der Züchtung der *Spirochæta pallida*, Berl. klin. Wchnschr., 1910, No. 42.

Sowade: Syphilitische Allgemeinerkrankung bei Kaninchen durch intrakardiale Kulturimpfung, Deutsch. med. Wchnschr., 1911, No. 15, p. 682.

Tomasczewski: Über den Nachweis der *Spirochæta pallida* bei tertiärer Syphilis, München. med. Wchnschr., 1906, No. 27, p. 1301; Über Impfungen in Affen maligner Syphilis, Berl. klin. Wchnschr., 1911, No. 20, p. 890.

Uhlenhuth and Mulzer: Die experimentelle Kaninchensyphilis, Arb. a. d. k. Gsndhtsamte., xxxiii, Part 1; Zur experimentellen Kaninchen und Affensyphilis, Berl. klin. Wchnschr., 1910, No. 25; Syphilitischen Allgemeinerkrankung bei Kaninchen, Deutsch. med. Wchnschr., 1911, No. 2, p. 51.

Volpino: Sulla colorazione delle *Spirochæta pallida* nelle sezioni di organi sifil., Gior. d. r. Accad. di med. di Torino., 1905, Nos. 11-12.

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CORRELATION OF THE HISTOLOGICAL AND CHEMICAL CHANGES IN THE SPLEEN DURING NECROSIS AND AUTOLYSIS.*

BY HARRY J. CORPER, M.D.

(From the Pathological Laboratory of the University of Chicago, and the Physiological Laboratory of the University of Illinois.)

PLATES 62–65.

The original aim of this work was to correlate the chemical changes taking place in tissues undergoing necrosis and autolysis *in vivo,* with the histological picture observed under the same conditions. To do this carefully, it was found desirable to use an organ which was of fairly uniform structure and of uniform chemical composition, and one which could be easily cut off completely from its blood supply without materially affecting the welfare of the animal to which it belonged. For these experiments the spleen was found to be ideal, as it is readily accessible, and its entire blood supply can be easily cut off by ligating its pedicle, after which the organ can be returned to the abdominal cavity in an aseptic condition, to be removed and studied after certain definite intervals.

By using large dogs, moreover, it was hoped that sufficient material could be obtained to make a complete chemical analysis. But the rapidity of the changes *in vivo,* the absorption of the products of tissue disintegration, and the variable pictures produced by numerous complicating and uncontrollable factors, soon showed that it would be necessary to study this organ under conditions in which the autolytic changes were practically free from the influence of such factors as organization, leucocytic infiltration, absorption, inhibition of autolysis by serum, etc.

Aseptic autolysis *in vitro* takes place under simpler conditions, but the histological picture resembles very closely that seen in

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autolysis *in vivo* in the areas farthest removed from the surrounding influences. From the chemical standpoint, also, there was a distinct advantage, for the results *in vitro* could be used to control those obtained *in vivo,* and without some such control, misinterpretation of the results obtained by autolysis *in vivo* would certainly follow.

The most important histological changes that occur during the autolysis that follows cell death are in the nucleus, and since these changes are produced chiefly by specific enzymes, it was thought advisable to study these enzymes and the products which they produce in the dog's spleen in the hope of gaining information which would better explain, at least from a chemical standpoint, some of the histological changes. The details of this part of the work are contained in a previous paper.

Historical.—Up to the present, I have found in the literature no report of a comparative study of the chemical and histological changes in an organ or tissue during autolysis or necrosis. Of the earlier observations on related points, Weigert (1) described the changes undergone by dead tissues, and noted that all cells that had undergone coagulation necrosis had lost their nuclei, but he said that not all cells which had lost their nuclei need necessarily have suffered this change.

Goldmann (2) studied organs kept aseptically in moist chambers at 37° C., and found that the spleen showed no macroscopical changes upon autolysis, but on microscopical examination, after eight days the pulp cells showed changes, while the nuclei in the Malpighian bodies were well preserved. After fourteen days, the trabeculæ were still distinctly differentiated from the pulp, and the nuclei of the Malpighian corpuscles were still retained, but in the remainder of the tissue the nuclei disappeared almost completely.

Klebs (3) observed that in the destruction of the nuclei by mycotic processes two forms of nuclear changes were seen, karyolysis and karyorrhexis. Schmaus and Albrecht (4) found that after ligation of the renal vessels the first change was pyknosis, followed by karyorrhexis, with numerous intermediate variations between this and necrobiosis.

According to Carrière and Vanverts (5) ligation of the pedicle of the spleen results in the death of the organ, which becomes converted into an encapsulated mass of purulent and caseous material with proliferation inward from the capsule. Cultures containing an innocuous colon bacillus, often associated with other harmless organisms, were obtained from the spleen of all the animals experimented upon, both before ligation of the vessels and after the destruction of the spleen was complete. To these organisms the authors attributed the caseous and purulent degeneration that occurred.

Davidsohn (6) transplanted spleens into mice, and later found only pigment rests remaining. Zervos (7) noted that spleens are absorbed in less than a

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year after transplantation. Lüdke (8) succeeded in transplanting a rabbit's spleen into the spleens of monkeys, and states that sections showed the included spleens well healed in, but he does not describe the appearances. Carrel (9) in his replantation experiments had accidentally an occlusion of the splenic artery in a dog, and noted that in about a year the spleen had completely disappeared Wells (10) observed that salt solution has a solvent action upon cytoplasm, but that otherwise the autolysis in salt solution proceeded at the same rate as in heated serum, and that splenic tissue after being killed by heat (100° C.) undergoes a very slow change in the body of the rabbit, being attacked only by invading leucocytes, while unheated spleen under the same conditions undergoes rapid autolytic changes with pyknosis, etc.

Although no complete study of the changes occurring in the proteins and nucleoproteins of the spleen during autolysis has been reported, a few observations concerning their constituents have been made. Levene (11) found as end products of autolysis of the spleen, alanine, leucine, aminovalerianic acid, aminobutyric acid, α pyrrolidine-carboxylic acid, phenylalanine, aspartic acid, tyrosine hypoxanthine, xanthine, thymine, and uracyl. Schumm (12) studied autolyzed leukemic spleens and observed a marked drop in the albumose content, and associated with this a threefold increase in non-coagulable nitrogen, which must have come from the coagulable part of the spleen substance as a result of autolysis. He identified as products of autolysis, lysine, leucine, tyrosine, paralactic acid, guanine, xanthine, and hypoxanthine.

Hedin (13) and his coworkers (14) found two distinct proteolytic enzymes (proteases) in the spleen, an α lieno-protease which acts chiefly in alkaline medium, and a β lieno-protease which acts in acid medium. Leathes (15) identified as products of the action of the proteolytic enzyme of the spleen of oxen in acid medium, leucine, tyrosine, aminovalerianic acid, aspartic acid, arginine, histidine, and lysine. The digested fluid also gave the tryptophane reaction, and spectroscopically hematin was found.

Cathcart (16) isolated the products of digestion of coagulated blood serum by α lieno-protease in an alkaline medium, and found histidine, inactive arginine, lysine, tryosine, leucine, alanine, aminovalerianic acid, α pyrrolidine-carboxylic acid, glutamic acid, phenylalanine, and ammonia. Aspartic acid was also probably present, it being formed in large amounts in the digestion of fibrin by the above enzyme. The most marked difference between the action of the two enzymes was in the nature of the arginine found, that obtained by Leathes being optically active.

The changes produced by autolysis in the individual constituents of the spleen have received but slight attention. In putrefaction, lecithin is decomposed into glycerophosphoric acid (17) and choline (18), the latter being further decomposed into methylamine, ammonia, carbonic acid, and methane. Choline is also produced in the autolysis (18) of lecithin, and Coriat believes that brain tissue contains an enzyme capable of producing this change. Meyer (19) found that within three to five weeks the amount of lecithin in pus is decreased by autolysis to about one third.

In autolysis of the liver, pancreas, and thymus the nuclein phosphorus sinks much faster than the lecithin phosphorus, both being converted into the inorganic phosphorus fraction (Morpurgo and Satta (20)).

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On account of the difficulty experienced in determining cholesterol quantitatively, only a few observations have been made upon the autolysis of this material. Carbone (21) and Waldvogel (22) believe (what is chemically impossible) that cholesterol originates from lecithin, the latter claiming to have established this by observations upon the digestion of lecithin by sterile liver juice. In experiments which extended over a period of forty-four days he says he obtained a decrease in lecithin and an increase in the fats, fatty acids, and cholesterol, and found a like increase on comparing normal livers with livers showing pathological changes after acute poisoning. Moore (23), however, failed to corroborate these observations in liver autolysis that lasted forty-two days at 37° C. under toluene. In his experiments he obtained 0.038 per cent. of cholesterol before, and 0.0372 per cent. after autolysis. In human splenic pulp he found no essential difference between the cholesterol content in a normal area (0.64 per cent.) and that in an anemic infarcted area (0.58 per cent.).

Practically no chemical study of the iron distribution in the tissues during autolysis has been made. Schmidt (24) found that in anemic infarcts the microchemical reaction for iron was negative or less than that in the surrounding sound tissues. The granular form of iron remaining was distributed over the entire tissue, but was thickest about and in the trabeculæ, which also showed a greater resistance to autolysis or necrosis. A diffuse iron test (Berlin blue and ammonium sulphide reactions) was obtained in the tissues containing granular iron, and Schmidt thinks a solution of iron has taken place, the iron being derived not only from the pigment but also from the entire hemosiderin. Such anemic infarcts often contain hematoidin needles in greater or lesser amounts; and not only is there no iron-containing pigment formed from the blood, but even the preformed iron is removed from the dead tissues. All ordinary embolic splenic infarcts are iron-free before other changes have occurred. Schmidt believes that the iron is not removed by these cells, but that it is destroyed (zerstört) at the site of the infarct. He does not believe that organic combination occurs which might make the microchemical test worthless. His conclusion is that hemosiderin disappears from the tissues on their death, possibly passing through the formation of soluble iron combinations (iron-lecithin), the character of which cannot as yet be stated. It has been suggested that the formation of organic acids in the infarct may explain this disappearance of iron (25).

Methods.—The methods of chemical analysis employed here are identical with those given in a previous paper (26) on the chemical composition of the spleen. Two series of experiments were carried out, those *in vitro* and those *in vivo*.

Before autolysis began in the experiments *in vitro*, samples were taken for the determination of moisture and for the study of the normal histology. Autolysis was carried out under toluene in 0.9 per cent. salt solution at room temperature after the tissue had been cut into small pieces, two to four millimeters square. At the

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termination of the autolysis, samples were taken for histological study and the autolytic processes were stopped by means of ten or more volumes of 95 per cent. alcohol in which the material was preserved in the ice chest until the analyses were undertaken.

In the experiments *in vivo,* absolute aseptic technique was used, and the largest dogs obtainable were employed. The pedicle of the spleen was doubly ligated by means of a silk thread, and, after varying intervals had elapsed, the spleen or what remained of it was removed and studied chemically and histologically as in the *in vitro* experiments.

Controls.—As controls on the experiments to be described, the results of chemical analyses and microscopical examinations of normal spleens are here given.

Chemical Composition of Normal Spleen.—Practically all the nuclein nitrogen was either insoluble, precipitable, or coagulable by alcohol. At most the soluble part was but a trace. About 24.1 per cent. of the total phosphorus existed in the form of phosphatids (lecithin, etc.), about 32 per cent. was soluble in water (inorganic phosphates, etc.) and about 40.1 per cent. was insoluble or coagulable (nucleoproteins and other organic coagulable and precipitable combinations). About 5.7 per cent. of the total nitrogen was soluble in water (peptones, amino acids, and ammonia compounds, purins, gelatin, etc.), and 94.3 per cent. insoluble (proteins, nucleoproteins, and other insoluble, precipitable, and coagulable organic nitrogen combinations).

Microscopical Appearance of Normal Spleen.—The trabeculæ were well defined and had nuclei which were distinct and elongated with rounded ends. The nuclei had definitely outlined borders and contained numerous small granules that stained with hematoxylin. The spleen pulp was fairly uniform throughout and had clear oval or round nuclei of various sizes. In nearly all cases these nuclei had a definite outline and contained small hematoxylin granules. In many cases the cytoplasm of individual cells could be distinctly made out, but the granular appearance of the ground substance made this impossible in places. The Malpighian corpuscles were well marked off and distinct and had well defined nuclei which lay close together, were deeply stained by hematoxylin, and contained a definite nucle-

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olus. Only rarely could the narrow layer of cytoplasm be made out in these cells. The capsule resembled the trabeculæ in appearance.

The description just given serves for the normal spleens A, B, and C, and for all of the spleens that were subsequently autolyzed *in vitro*.

I. EXPERIMENTS IN VITRO.

Spleen D Autolyzed for Seventeen Hours (Figures 1 and 2).—This spleen was large and before autolysis it had a moist weight of 62.5 gm. and a moisture content of 78.09 per cent. Its dry weight was, therefore, 13.7 gm.

Chemical Analysis of Spleen D after Autolysis for Seventeen Hours.—In the ether-soluble fraction the total material weighed 1.7938 gm., or 13.09 per cent. of the dry weight. The part of this that was insoluble in absolute alcohol weighed 0.0994 gm. and contained 0.0017 gm. of phosphorus (after Neumann oxidation). The cholesterol figure was valueless.

In 1 gm. of dry tissue the lecithin phosphorus weighed 0.00187 gm., which, estimated as lecithin, would amount to 0.0482 gm., and the non-lecithin phosphorus in this fraction weighed 0.00013 gm.

Water-soluble Fraction of Spleen D.—One gm. of dry tissue yielded 0.0103 gm. of nitrogen, of which 0.0021 gm. was precipitated by tannic acid and 0.0082 gm. was not precipitated by tannic acid.

In 1 gm. of dry tissue the water-soluble phosphorus weighed 0.00287 gm.

It was doubtful whether even a trace of purin was present in the water-soluble extractives.

Insoluble Tissue Residue.—One gm. of tissue residue yielded 0.0059 gm. of iron, 0.00689 gm. of sulphur, and 0.00484 gm. of phosphorus. As a result of analyzing about 5 gm. of dry material, 0.00464 gm. of purin nitrogen was found per gram of dry insoluble tissue residue. The total nitrogen in 1 gm. of tissue was 0.1635 gm. In determining this, duplicate analyses, each of 0.25 gm. of tissue, were made.

Microscopical Findings in Spleen D after Autolysis for Seventeen Hours.—After autolysis the trabeculæ and capsules did not differ visibly in appearance from the normal. The splenic pulp, however, showed definite changes. The cells appeared to be fewer in number (few nuclei were present) and stood out with distinct cell boundaries against the reticular framework of the pulp. Their cytoplasm stained well with eosin and contained numerous small vacuoles. The nuclei in most cases were still definitely marked off from the cytoplasm, but stained deeply with hematoxylin and in most cases were homogeneous (pyknosis). A few cells were found in which the nuclei were fragmented, and some nuclei were shaped like crenated red cells. In some cells no nucleus could be seen. The Malpighian corpuscles also showed changes. Cell boundaries were more definite than normally and most of the nuclei stained homogeneously blue. Some nuclei showed fragmentation (karyorrhexis) and some appeared as large rings.

Spleen E Autolyzed for Thirty Hours (Figures 3 and 4).—Before autolysis this spleen had a moist weight of 62.5 gm. and a moisture content of 76.54 per cent. The dry weight was, therefore, 14.67 gm.

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Chemical Analysis of Spleen E after Autolysis for Thirty Hours.—In the ether-soluble fraction the total material weighed 2.349 gm., or 16.01 per cent. The part of this that was insoluble in absolute alcohol weighed 0.0280 gm., and contained 0.001 gm. of phosphorus (after Neumann oxidation). The figures obtained for cholesterol were valueless. The lecithin phosphorus found in 1 gm. of dry tissue weighed 0.00228 gm. which, figured as lecithin, amounts to 0.0587 gm. The non-lecithin phosphorus found in this fraction weighed 0.000014 gm.

Water-soluble Fraction.—In 1 gm. of dry tissue the nitrogen precipitated by tannic acid weighed 0.0079 gm., and the nitrogen not precipitated by tannic acid weighed 0.0268 gm. The total nitrogen in the water-soluble fraction was, therefore, 0.0347 gm. The phosphorus in the water-soluble fraction of 1 gm. of dry tissue weighed 0.0054 gm., and the purin nitrogen determined in 60 per cent. of the total water-soluble extractive, amounted to 0.00039 gm. per gram of dry tissue.

Insoluble Tissue Residue.—In 1 gm. of tissue there was 0.0078 gm. of iron, 0.0078 gm. of sulphur, and 0.006 gm. of phosphorus; and in 1 gm. of dry tissue residue there was 0.006 gm. of purin nitrogen. For the last determination a 4 gm. sample was examined. The total nitrogen was 0.1610 gm. per gram of tissue. In estimating this, duplicate analyses were made, each upon 0.25 gm. of tissue residue.

Microscopical Findings in Spleen E after Autolysis for Thirty Hours.—The definition of the trabeculæ and capsule was not as clear as in spleen D, but the nuclei appeared to be more distinct, not differing much from normal, however. The splenic pulp had suffered a further marked change, and appeared granular with numerous small hematoxylin-stained dots on a diffuse bluish tinted field. The nuclei seemed to have undergone disintegration and fragmentation (karyorrhexis), leaving hematoxylin-staining round and oval globules of various sizes and occasionally a distinct nucleus. The cytoplasm of the cells takes a bluish pink tint, and in some cases these cells appeared merely as shadows.

The Malpighian corpuscles stood out distinctly with their nuclei intact, but in shape some of the cells resembled crenated red corpuscles. The cytoplasm of these cells seemed to have increased in amount and in many cases presented a definite border.

Spleen F Autolyzed for Fifty-three Hours (Figures 5 and 6). Chemical Analysis.—Three spleens which before autolysis had a moist weight of 30, 31.5, and 23.5 gm., and a moisture content of 74.25 per cent., 77.93 per cent., and 77.04 per cent. respectively, were united and used. Their total dry weight was 20.07 gm.

Ether-soluble Material.—The dry weight of this fraction was 2.3085 gm. or 11.5 per cent. Of this the part insoluble in absolute alcohol weighed 0.0787 gm., and contained 0.0093 gm. of phosphorus (after Neumann oxidation). The cholesterol figure obtained was valueless. The lecithin phosphorus found in 1 gm. of dry tissue weighed 0.00157 gm., which, figured as lecithin, amounted to 0.0404 gm. The non-lecithin phosphorus found in this fraction weighed 0.00019 gm.

Water-soluble Fraction.—In 1 gm. of the dry tissue the nitrogen precipitated by tannic acid weighed 0.0056 gm., and the nitrogen not precipitated by tannic acid weighed 0.0319 gm. The total nitrogen in the water-soluble fraction was,

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therefore, 0.0375 gm. The phosphorus in the water-soluble fraction of 1 gm. of dry tissue weighed 0.0063 gm., and the purin nitrogen determined in 0.7 gm. of the total water-soluble extractives amounted to 0.00098 gm. per gram of dry tissue.

Insoluble Tissue Residue.—One gm. of tissue residue yielded 0.00437 gm. of iron, 0.00656 gm. of sulphur, and 0.00416 gm. of phosphorus. One gm. of dry tissue residue (a 5.5 gm. sample was analyzed) yielded 0.00448 gm. of purin nitrogen. The total nitrogen was 0.1610 gm. per gram of tissue residue, the analyses being made in duplicate, each on 0.25 gm. of tissue residue.

Microscopical Findings in Spleen F after Autolysis for Fifty-three Hours.—The trabeculæ and capsule could still be distinctly differentiated from the spleen pulp, but the nuclei in the trabeculæ were decreased in number and size, solution seeming to have taken place at the borders, so that the nuclei appeared narrow and long. Well formed nuclei, however, could still be seen in some parts of the trabeculæ. Pyknosis was not clearly observed. The pulp had a homogeneous, granular appearance. Many of these granules or droplets stained conspicuously with hematoxylin. Some nuclei were still visible in the pulp, a few still staining slightly, but the majority remained merely as shadows.

The Malpighian corpuscles could no longer be clearly differentiated from the pulp, but in their stead, large numbers of shadows that stained faintly with hematoxylin were seen. These were aggregated especially closely about the blood vessels and trabeculæ. No intact nuclei were found.

Spleen G after Autolysis for Five Days. Chemical Analysis.—Five spleens, which before autolysis had a moist weight of 10 gm., 20 gm., 15.5 gm., 11 gm., and 45.5 gm., and a moisture content of 77.47 per cent., 76.65 per cent., 76.65 per cent., 79.11 per cent., and 77.21 per cent. respectively, were united and used. Their total dry weight was 23.21 gm.

Ether-soluble Fraction.—The total material weighed 2.6644 gm., or 11.47 per cent. Of this the absolute alcohol-insoluble part weighed 0.0654 gm. and contained 0.00178 gm. of phosphorus (after Neumann oxidation). The cholesterol figures obtained were of no value. The lecithin phosphorus in 1 gm. of dry tissue weighed 0.0014 gm., which, figured as lecithin, amounted to 0.03627 gm. The non-lecithin phosphorus in this fraction weighed 0.00009 gm.

Water-soluble Fraction.—In 1 gm. of dry tissue the nitrogen precipitated by tannic acid weighed 0.00505 gm., and the nitrogen not precipitated by tannic acid weighed 0.02998 gm. The total nitrogen in the water-soluble fraction was, therefore, 0.03503 gm. The phosphorus in the water-soluble fraction of 1 gm. of dry tissue weighed 0.0057 gm. The purin nitrogen in this fraction was not determined.

Insoluble Tissue Residue.—One gm. of tissue residue yielded 0.0075 gm. of iron, 0.00894 gm. of sulphur, and 0.0041 gm. of phosphorus. One gm. of dry tissue residue (about 6 gm. were analyzed) yielded 0.00377 gm. of purin nitrogen. The total nitrogen amounted to 0.1622 gm. per gram of tissue residue, the analyses being made in duplicate, each on about 0.25 gm. of tissue residue.

Hausmann Fractions of Proteins in Tissue Residue.—The following figures were obtained from 1 gm. of dry tissue residue. These figures are the mean of duplicate analyses.

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Amid nitrogen	= 0.01311 gm. nitrogen.
Humus nitrogen	= 0.00678 gm. nitrogen.
Diamino nitrogen	= 0.03897 gm. nitrogen.
Monamino nitrogen	= 0.1031 gm. nitrogen.
Total nitrogen figured	= 0.16196 gm. nitrogen.

Microscopical Findings in Spleen G after Autolysis for Five Days.—The trabeculæ and capsule were still differentiated, but not as clearly as in F, and a few nuclei were found which stained deeply with hematoxylin, no fragmentation being visible.

The splenic pulp and Malpighian corpuscles were a mass of diffusely stained granular material, with a slightly greater concentration of hematoxylin-stained granules where the Malphigian corpuscles previously existed. In the pulp proper most of the hematoxylin-stained globules have entirely disappeared, leaving a granular ground substance made up in places of what appear to be the shadows of previously existing cells. On close inspection the nuclear boundaries may be seen in many cases, and in some cases the cell boundaries also. Where the Malpighian corpuscles were formerly located there is a greater number of these cell shadows. The endothelial cells on the surface of the capsule seemed to be perfectly preserved.

Spleen H after Autolysis for Six Days (*Figures 7 and 8*). *Chemical Analysis.*—Three spleens, two having together a moist weight of 67 gm., and the third a moist weight of 42 gm., with a moisture content of 77.98 per cent. and 77.72 per cent. for the first two, and 77.17 per cent. for the third, were united and used. The total dry weight was 24.51 gm.

Ether-soluble Fraction.—The total material soluble in ether weighed 2.887 gm., or 11.78 per cent. Of this the part insoluble in absolute alcohol weighed 0.0364 gm. and contained 0.00153 gm. of phosphorus (after Neumann oxidation). Cholesterol found in 1 gm. of dry tissue weighed 0.02162 gm. In making this determination one half of the total of ether-soluble material was used for analysis. The lecithin phosphorus in 1 gm. of dry tissue weighed 0.00133 gm., which, figured as lecithin, amounted to 0.0343 gm. The non-lecithin phosphorus in this fraction weighed 0.000066 gm.

Water-soluble Fraction.—In 1 gm. of dry tissue the nitrogen precipitated by tannic acid weighed 0.00476 gm., and the nitrogen not precipitated by tannic acid weighed 0.03680 gm. The total nitrogen in the water-soluble fraction of 1 gm. was, therefore, 0.04156 gm. The phosphorus in the water-soluble fraction of 1 gm. of the dry tissue weighed 0.00691 gm. and the purin nitrogen determined in 0.6 gm. of the total water-soluble extractives amounted to 0.00072 gm.

Insoluble Tissue Residue.—One gm. of tissue residue yielded 0.003285 gm. of phosphorus, and 1 gm. of dry tissue residue (about 5 gm. were used) yielded 0.00315 gm. of purin nitrogen.

Hausmann Fractions of Proteins in Tissue Residue.—The figures obtained from 1 gm. of dry tissue residue follow. These figures are the mean of duplicate analyses.

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Amid nitrogen	= 0.01247 gm. of nitrogen.
Humus nitrogen	= 0.00767 gm. of nitrogen.
Diamino nitrogen	= 0.04158 gm. of nitrogen.
Monamino nitrogen	= 0.10660 gm. of nitrogen.
Total nitrogen	= 0.16832 gm. of nitrogen.

Microscopical Findings in Spleen H after Autolysis for Six Days.—The autolysis had gone even a step farther here than in spleen G, so that, with the exception of an occasional nucleus here and there, practically none could be seen even in the trabeculæ. Otherwise the section resembled that of spleen G, the pulp merely showing shadows of cells with no definite stained nuclei. The form of the nuclei, however, was apparently still visible in some cells.

II. EXPERIMENTS IN VIVO.

Spleen I after Autolysis for Two Days. Chemical Analysis.—A single spleen removed from a dog two days after the pedicle had been completely tied off had a moist weight of 121 gm. and a moisture content (determinations made in duplicate) of 83.84 per cent., making a total dry weight of 19.86 gm.

Ether-soluble Fraction.—The total material weighed 2.1629 gm., or 10.88 per cent. of the dry weight. Of this the part insoluble in absolute alcohol weighed 0.2442 gm. and contained 0.0013 gm. of phosphorus (after Neumann oxidation). The cholesterol found in 1 gm. of dry tissue weighed 0.02175 gm. In making this determination one half of the total material soluble in ether was used for analysis. The lecithin phosphorus found in 1 gm. of the dry tissue weighed 0.000066 gm., which, figured as lecithin, amounted to 0.0017 gm. The non-lecithin phosphorus found in this fraction weighed 0.000023 gm.

Water-soluble Fraction.—In one gm. of dry tissue the nitrogen precipitated by tannic acid weighed 0.0009 gm., and the nitrogen not precipitated by tannic acid weighed 0.00649 gm. The total quantity of nitrogen in the water-soluble fraction of 1 gm. was, therefore, 0.00739 gm. The phosphorus in the water-soluble fraction of 1 gm. of dry tissue weighed 0.00085 gm. An uncertain trace of purin was found in this fraction, three fifths of the total extractives being used for analysis.

Insoluble Tissue Residue.—One gm. of dry tissue residue yielded 0.0070 gm. of iron, 0.00785 gm. of sulphur, and 0.00153 gm. of phosphorus. One gm. of dry tissue residue (about 5 gm. samples being used for duplicate analyses, which agreed well) yielded 0.00056 gm. of purin nitrogen.

Hausmann Fractions of Proteins in Tissue Residue.—The mean figures obtained from duplicate analyses of 1 gm. of dry tissue residue are as follows:

Amid nitrogen	= 0.01197 gm. of nitrogen.
Humus nitrogen	= 0.00784 gm. of nitrogen.
Diamino nitrogen	= 0.03605 gm. of nitrogen.
Monamino nitrogen	= 0.10230 gm. of nitrogen.
Total nitrogen figured	= 0.15816 gm. of nitrogen.

Spleen I after Autolysis for One Day.—The spleen was obtained from a 50 kilo dog, in which the pedicle had been ligated two days previously. The spleen

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was soft and of a greenish appearance, but its consistence was such that it could still be cut without disintegration.

On microscopical examination the trabeculæ, from which all vestiges of nuclei had disappeared, could still be differentiated from the pulp, but the staining qualities were about the same in both, affinity for hematoxylin having been lost. The surface of the capsule, however, was invaded superficially by leucocytes and by proliferating connective tissue cells, and probably by endothelial cells, while in the deeper part of the capsule these were absent, only shadow-like tissue forms being seen. The splenic pulp was one mass of granular debris with here and there a stray leucocyte. These were seen especially near the capsule. In appearance the pulp resembled that of spleen G, except that the form of the cells seemed to be better preserved and were seen more distinctly. In certain areas in the pulp, pigment-laden cells containing yellowish and brownish granules were seen. These were especially noticeable near the capsule.

Spleen K after Autolysis for Ten Days.—A dog weighing 24.8 kilos was used for this experiment, the spleen pedicle being tied off by about ten double ligatures. The rectal temperature on the day of the operation and during the succeeding days ranged between 101.9° F. and 104° F. No ill effects were noticed except those consequent to the ether anesthesia. On the tenth day the animal was chloroformed and the post-mortem examination revealed an intensely congested omentum which was adherent to the intestines in several places, and in the peritoneal cavity was seen a thick reddish exudate. The spleen was found in an area of intense congestion and adhesions in the left upper posterior part of the peritoneal cavity. It was encapsulated by a fibrous tissue capsule which when opened revealed a central greenish semi-liquid mass weighing about 6.5 gm. and having a moisture content of 80.81 per cent. (average of duplicate determinations). The left kidney appeared slightly congested and there were small petechiæ on the left diaphragmatic wall. No chemical analysis of this small amount of material was attempted.

Microscopical examination revealed a typical necrotic tissue with no nuclei and only the semblance of the previously existing trabeculæ and splenic pulp. No marked leucocytic invasion was noted, except at the periphery of what appeared to be the remnants of the capsule, where a few leucocytes, connective tissue, and endothelial cells were found. The pulp was diffusely granular and in places were seen what appeared to be the shadows of cells with here and there areas that contained masses of large, rod-shaped organisms.

Spleen L after Autolysis for Twenty Days.—As in previous experiments the splenic pedicle was doubly ligated in a dog weighing 25.8 kilos, and the daily rectal temperature in this animal ranged between 101.2° and 103.8° F. Post-mortem examination on the twentieth day revealed a reddish and congested omentum and, in the peritoneal cavity, a small amount of thick reddish exudate which was less in amount than that seen in the ten day experiment. The spleen remnant was found with omental adhesions in a mass of connective tissue. Incision through this capsule revealed a small amount of faintly reddish creamy liquid, which could not be studied chemically. It had, however, a moisture content of 82.72 per cent. (average of duplicate analyses).

Spleen M Autolyzed for Forty Days.—A dog weighing 25.9 kilos was used. The splenic pedicle was ligated as in previous experiments, and the rectal tem-

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perature (taken at intervals during the next forty days), ranged between 101.3° and 105.8° F. On the fortieth day the dog appeared to be in perfect health.

After it had been chloroformed, post-mortem examination revealed no exudate in the peritoneal cavity, but there was still a slight congestion of the omentum. The spleen remnant was completely encapsulated and on section only the dense capsule with a small reddish center was found. No chemical examination could be made upon this material, but its moisture content was found to be 82.53 per cent. (the average of duplicate determinations.)

Upon microscopical examination the splenic remnant was found to be made up of typical scar tissue, interspersed with small areas containing pigment-laden cells. The connective tissue was irregularly arranged and was composed of spindle-shaped cells. In some places the cellular elements predominated, in others the intercellular material. The pigment-laden cells were of large size, contained a small nucleus, and in their cytoplasm was seen a large amount of brownish yellow granular pigment. In one section which appeared to contain the former capsule of the spleen there was a more regular arrangement of the various types of connective tissue, and internal to this were found a large number of large phagocytic pigment cells.

The results of the foregoing analyses are summarized in tables II and III.

The results were as follows:

1. There was a gradual but definite decrease in the total amount of the ether-soluble materials. The amount of cholesterol did not change to an appreciable extent, but the lecithin and phosphorus contents decreased proportionately to the stage of autolysis.

2. There was a gradual but definite and proportionate increase in the total water-soluble nitrogen. The main regular increase was in the fraction not precipitated by tannic acid (polypeptids and amino acids). In the purins which had been split out of the nucleins by autolytic enzymes the increase was slight but regular, but in the fraction precipitated by tannic acid there was no appreciable change.

3. There was an increase in the water-soluble phosphorus which progressed with autolysis, the addition being no doubt partly from the phosphorus set free through autolysis, but mainly from disintegration of nucleins.

4. There was a gradual but regular decrease in the phosphorus contained in the insoluble tissue residue. This was due to the nuclear material going into solution. The iron and sulphur content of this fraction did not reveal any consistent change during autolysis.

5. There was a gradual decrease in the purin nitrogen contained in the insoluble tissue residue (nuclein disintegration).

6. There was a slight but gradual decrease in the total nitrogen

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TABLE I.

Changes Produced in the Composition of the Spleen during Autolysis.

Fractions.	Divisions of fractions.	Per cent. of one gram of the original moisture-free tissue.								In vivo.
		Normal spleens.			Autolyzed spleens. In vitro.					
		A	B	C	D	E	F	G	H	I
Ether-soluble fraction	Total	15.47	11.66	15.11	13.09	16.01	11.50	11.47	11.78	10.88
	Absolute alcohol-soluble	13.82	9.57	14.05	12.37	15.82	11.11	11.19	11.63	9.65
	Absolute alcohol-insoluble	1.65	2.09	1.06	0.725	0.19	0.39	0.28	0.15	1.23
	Phosphorus content of absolute alcohol-insoluble	0.048	0.042	0.017	0.012	0.007	0.046	0.007	0.006	0.006
	Cholesterol		1.504						2.16	2.17
	Per cent. of ether-soluble as cholesterol		15.07						18.59	22.52
	Lecithin	6.94	Lost	6.21	4.82	5.86	4.03	3.62	3.43	0.17
	Per cent. of lecithin ether-soluble	50.23	Lost	44.23	39.05	37.05	36.36	32.41	29.49	1.76
	Lecithin phosphorus	0.269		0.241	0.187	0.227	0.156	0.141	0.133	0.006
	Non-lecithin phosphorus	0.050	Lost	0.0147	0.0131	0.0014	0.0187	0.009	0.006	0.0023
Water-soluble fraction	Nitrogen not precipitated by tannic acid	0.396	0.392	0.337	0.821	2.678	3.187	2.998	3.68	0.649
	Nitrogen precipitated by tannic acid	0.576	0.411	0.119	0.212	0.792	0.563	0.504	0.476	0.089
	Purin nitrogen	0.00	0.00	0.00	0.00	0.038	0.097	Lost	0.072	Trace
	Per cent. of total purin nitrogen	0.00	0.00	0.00	0.00	9.734	27.41	Lost	28.02	0.00
	Total nitrogen	0.972	0.803	0.456	1.033	3.47	3.75	3.50	4.156	0.738
	Phosphorus	0.518	0.345	0.269	0.287	0.543	0.632	0.570	0.691	0.085
Insoluble tissue residue	Iron	0.265	0.370	0.983	0.468	0.464	0.253	0.481		0.54
	Sulphur	0.533	0.581	0.600	0.547	0.464	0.380	0.574		0.606
	Phosphorus	0.637	0.381	0.393	0.345	0.356	0.241	0.262	0.194	0.118
	Purin nitrogen	0.261	0.238	0.345	0.369	0.357	0.259	0.241	0.186	0.041
	Total nitrogen	10.96	13.34	13.13	13.02	9.63	9.34	10.40	9.57	12.21

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TABLE II.

Changes Produced in the Nitrogen and Phosphorus Distribution of the Spleen during Autolysis.

Fraction.	Divisions of fractions.	Per cent. of the total nitrogen or phosphorus of the original tissue.								
		Normal spleens.			Autolyzed spleens. In vitro.					In vivo.
		A	B	C	D	E	F	G	H	I
Ether-soluble fraction	Absolute alcohol-insoluble phosphorus	3.17	4.11	1.91	1.48	0.61	4.22	0.776	0.606	3.07
	Lecithin phosphorus	17.69		25.75	22.12	20.04	14.31	14.24	12.93	3.02
	Non-lecithin phosphorus	3.29		1.57	1.54	0.123	1.70	0.916	0.636	1.073
Water-soluble fraction	Nitrogen not precipitated by tannic acid	3.32	2.77	2.48	5.84	20.44	24.35	21.57	26.83	5.01
	Nitrogen precipitated by tannic acid	4.83	2.91	0.879	1.51	6.04	4.30	3.63	3.46	0.69
	Purin nitrogen	0.00	0.00	0.00	Trace	0.294	0.748		0.527	Trace
	Phosphorus	34.03	33.09	28.76	33.96	47.83	57.71	57.66	67.05	38.86
Insoluble tissue residue	Phosphorus	41.81	36.51	41.99	40.81	31.41	22.03	26.51	18.82	53.98
	Purin nitrogen	2.19	1.68	2.54	2.62	2.72	1.98	1.73	1.35	0.318
	Total nitrogen	91.89	94.36	96.70	92.66	73.53	71.41	74.82	69.69	94.34

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TABLE III.

Spleens.	Per Cent. of Total Nitrogen in Extracted Residue.			
	Normal.	In vitro 5 days.	In vitro 6 days.	In vivo 2 days
	C	G	H	I
Amid nitrogen	8.60	8.09	7.40	7.57
Humus nitrogen	5.76	4.19	4.55	4.96
Diamino nitrogen	21.87	24.05	24.68	22.78
Monamino nitrogen	63.71	63.65	63.37	64.65
Total per cent.	99.94	99.98	100.00	99.96

contained in the insoluble tissue residue which, divided into the Hausmann fractions, did not reveal any appreciable difference in the nitrogen distribution in the protein of the tissue residue before or after autolysis.

Autolysis in Vivo.—The results were as follows:

1. There was a decrease in the ether-soluble fraction, which was more marked than the decrease after autolysis *in vitro*. In the phosphatid phosphorus there was a great decrease which was not associated with an increase in the water-soluble phosphorus, presumably because of diffusion of the soluble products of autolysis. As far as could be ascertained there was, however, no appreciable change in the cholesterol determinations. This was discussed in an earlier paper on cholesterol determinations (26).

2. In the total water-soluble nitrogen there was no appreciable increase, but there was a doubling of the nitrogen not precipitated by tannic acid, a decrease in the nitrogen precipitated by tannic acid, and an absence of purin nitrogen.

3. In the water-soluble phosphorus there was a marked decrease due to diffusion.

4. The phosphorus contained in the insoluble tissue residue decreased, but the iron and sulphur content of this fraction were normal, behaving as they did after autolysis *in vitro*.

5. A much greater decrease was noted in the insoluble purin nitrogen.

6. In the total nitrogen or in the Hausmann fractions of the insoluble tissue residue after autolysis, there was no appreciable change.

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SIGNIFICANCE OF CHEMICAL CHANGES OCCURRING IN AUTOLYSIS, WITH SPECIAL REFERENCE TO THE HISTOLOGICAL CHANGES OCCURRING COINCIDENTALLY.

In drawing conclusions from microscopical examinations of a tissue we can, at most, notice only the superficial changes, and the main ones occur in the nucleus. With the aid of modern chemistry we are, however, enabled to investigate the cell further and to associate what we see histologically with what actually happens to some, at least, of the chemical components of tissues. As methods of biochemical analysis are still far from perfect, we must be satisfied with a study of such simple components as can be accurately estimated chemically.

When the pathologist finds microscopical changes in a tissue, he associates these with what he has previously seen, but he has no knowledge as to how much of the actual tissue constituents has been removed in the process, whether all are still present but in a changed form, or whether the greater part has been removed, leaving only the mere framework or skeleton; in other words, he has no means of knowing what percentage of the protein, nucleoprotein, lecithin, etc., of the original tissue still exists as such when certain microscopical changes are observable.

A few of these questions are answered by the results of this investigation, and will be taken up here by considering the changes in the chemical constituents which are associated with the microscopically visible pathological changes.

In the normal spleen the nucleins are practically all in forms which are insoluble, coagulable, or precipitable. In autolysis *in vitro* they remain thus in the first stages, even when a high grade of pyknosis is visible microscopically. When karyorrhexis and karyolysis are first seen, a small percentage (about 10 per cent.) of the nuclein nitrogen has become soluble in the form of purin bases, but when karyolysis is completed so that no nuclei remain that stain, only about 28 per cent. of the nuclein nitrogen is soluble (purin bases). At this point an approximate equilibrium has apparently been reached, and after this as autolysis progresses there is only a slow increase in the soluble nuclein nitrogen. Here we find an unexpected fact, that when all visible nuclear structures are lost, three

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fourths of the nucleins are still demonstrable. Although nuclein disintegration goes at first hand in hand with karyolysis, it is not completed with the completion of karyolysis.

In vivo, however, the conditions are different, and the nuclein nitrogen may be reduced to a very small percentage by the time karyolysis is complete, the nuclein nitrogen being removed almost as fast as it is liberated. In autolysis *in vivo* diffusion into the surrounding body fluids probably takes place, this apparently being shown by the fact that no soluble purin nitrogen was found in the analysis of the spleen autolyzed *in vivo*.

In the normal spleen about 24 per cent. of the total phosphorus exists in the form of phosphatids. After autolysis *in vitro* only a slight decrease in the phosphatid content has occurred even when pyknosis is marked. However, when karyorrhexis and karyolysis are conspicuous features of the tissue changes, the phosphatid phosphorus has decreased to about 20 per cent. of the total phosphorus, and at the completion of karyolysis the phosphatid phosphorus amounts to about 16 per cent. of the total. Finally, when nuclear destruction is histologically complete, the phosphatid phosphorus has decreased to about 13.6 per cent. of the total phosphorus, or about one half to two thirds the amount contained in the normal spleen.

In vivo when histological changes in the spleen had practically come to an end the phosphatid phosphorus had decreased to about 4 per cent. of the total phosphorus still present, and this percentage would have been far lower if it had been calculated with reference to the total phosphorus originally present in the normal spleen.

In the normal spleen about 40 per cent. of the total phosphorus exists in the coagulable and precipitable (insoluble) tissue materials (nucleins, proteins, and organic phosphorus compounds) other than the phosphatids, which were discussed above. After autolysis *in vitro* when pyknosis is prominent there is practically no decrease in this percentage, but with the appearance of karyorrhexis and karyolysis there is a drop to 31.4 per cent. of the total phosphorus, and at the termination of the lytic process the phosphorus content has fallen to 22 per cent. of the total, and decreases to about 19 per cent. when the nuclear destruction is microscopically complete.

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In the normal spleen only about 5.7 per cent. of the total nitrogen is in the water-soluble fraction (in the form of ammonium compounds, amino acids, peptones, purin bases, gelatin, etc.), while about 94.3 per cent. of the nitrogen is in an insoluble, coagulable, or precipitable form (proteins, nucleoproteins, and other insoluble organic nitrogen combinations). After autolysis *in vitro* when pyknosis is prominent some of the nitrogen has been changed to the soluble form, so that 7.4 per cent. is soluble. When karyorrhexis and karyolysis are conspicuous the water-soluble nitrogen has markedly increased, so that 26.5 per cent. of the total nitrogen is in this form. The increase, however, is due mainly to amino acids, peptones, and simple nitrogen derivatives, as the part precipitated by tannic acid shows practically no increase. When karyolysis is nearly complete, the soluble nitrogen has increased to 28.6 per cent. of the total nitrogen, and after the microscopical tissue changes have practically ceased the soluble nitrogen amounts to 30.3 per cent., leaving about 69.7 per cent. of the total nitrogen in the insoluble form (precipitable and coagulable by alcohol). However, with autolysis *in vivo* diffusion tends to keep the soluble nitrogen down to about the normal percentage, so that when microscopical tissue disintegration is practically at an end there is but 5.7 per cent. of the total nitrogen in the soluble form, this amount being identical with that in the normal spleen.

SUMMARY.

The most marked chemical changes in a disintegrating spleen are noticed during the period when karyorrhexis and karyolysis are the characteristic features. When pyknosis alone is prominent almost all of the several constituents are in the coagulable or precipitable fraction, just as they are in the original normal tissue. After karyolysis is practically complete the coagulable and precipitable elements of the tissue still gradually decrease in amount, but the rate is far below that occurring during karyorrhexis and karyolysis. Moreover, even when microscopical nuclear disintegration is practically at an end, the chemical process of disintegration is still going on, but very slowly. Finally, when nuclear structures can no longer be seen in the spleen, about 72 per cent. of the nuclein

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nitrogen, about 50 per cent. of the original insoluble phosphorus, and 70 per cent. of the original insoluble nitrogen, are still in the insoluble form (precipitable and coagulable by alcohol), while about two thirds of the phosphatid phosphorus remains in complex organic combinations.

BIBLIOGRAPHY.

1. C. Weigert, *Deutsch. med. Wchnschr.*, 1885, xi, 747, 780, 795, 813. Translated by J. P. Crozer Griffith, *Med. Times*, 1885, xvi, 810.
2. E. E. Goldmann, *Fortschr. d. Med.*, 1888, vi, 889.
3. Klebs, cited by G. Arnheim, *Virchows Arch. f. path. Anat.*, 1890, cxx, 367.
4. H. Schmaus and E. Albrecht, *Virchows Arch. f. path. Anat.*, 1895, cxxxviii, Suppl., 1.
5. C. Carrière and J. Vanverts, *Arch. d. méd. expér.*, 1899, xi, 498.
6. Davidsohn, *Verhandl. d. deutsch. path. Gesellsch.*, 1904, vii, 39.
7. Zervos, *Grèce méd.*, 1910, xii, 1.
8. Lüdke, *München. med. Wchnschr.*, 1909, lvi, 1359.
9. A. Carrel, *Jour. Exper. Med.*, 1910, xii, 146.
10. H. G. Wells, *Jour. Med. Research*, 1906, xv, 149.
11. P. A. Levene, *Am. Jour. Physiol.*, 1904, xi, 437; 1904–5, xii, 276.
12. O. Schumm, *Beitr. z. chem. Phys. u. Path.*, 1903, iii, 576.
13. S. G. Hedin, *Jour. Physiol.*, 1903, xxx, 155.
14. S. G. Hedin and S. Rowland, *Ztschr. f. physiol. Chem.*, 1901, xxxii, 341.
15. J. B. Leathes, *Jour. Physiol.*, 1902, xxviii, 360.
16. E. P. Cathcart, *Jour. Physiol.*, 1905, xxxii, 299.
17. K. Hasebroek, *Ztschr. f. physiol. Chem.*, 1888, xii, 148.
18. I. Coriat, *Am. Jour. Physiol.*, 1904–5, xii, 353.
19. E. Meyer, *Verhandl. d. deutsch. path. Gesellsch.*, 1903, vi, 92.
20. Morpurgo and Satta, *Arch. ital. de biol.*, 1908, xlix, 375.
21. T. Carbone, *Arch. ital. de biol.*, 1896–7, xxvi, 279.
22. Waldvogel and Mette, *München. med. Wchnschr.*, 1906, liii, 402.
23. F. Craven Moore, *Med. Chron.*, 1907–8, xlvii, 204.
24. M. B. Schmidt, *Verhandl. d. deutsch. path. Gesellsch.*, 1908, xii, 271.
25. H. G. Wells, *Arch. Int. Med.*, 1911, vii, 721.
26. H. J. Corper, *Jour. Biol. Chem.*, 1912, xi, 27, 37.

EXPLANATION OF PLATES.

PLATE 62.

FIGS. 1 and 2. Dog spleen after autolysis for seventeen hours. Microscopically pyknosis is prominent and there is some nuclear fragmentation. Practically all the nuclein nitrogen (about 2.6 per cent. of the total nitrogen) is still in the insoluble and coagulable form, with possibly a trace that is soluble. The phosphorus in the form of phosphatids has, however, suffered a slight decrease in amount, being now about 23.6 per cent. of the total phosphorus. The water-soluble phosphorus has increased slightly (part of this being derived from the

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phosphatid fraction) and is now 34 per cent. of the total. The insoluble phosphorus remains about the same, 40.8 per cent. of the total. The water-soluble nitrogen has increased slightly over normal, being 7.4 per cent. of the total (a difference of 1.7 per cent., while the insoluble nitrogen has suffered a corresponding decrease, being now 92.6 per cent. of the total nitrogen, a difference of 1.7 per cent.

PLATE 63.

FIGS. 3 and 4. Dog spleen after autolysis for thirty hours. Microscopically karyorrhexis and karyolysis are conspicuous, especially in the pulp, and 9.7 per cent. of the nuclein nitrogen has become soluble, that is, 0.3 per cent. of the total nitrogen is in the form of soluble purin nitrogen, while 90.3 per cent. of the purin nitrogen, or 2.7 per cent. of the total, is still insoluble. The phosphorus in the phosphatid form has suffered a still further decrease, now amounting to 20.2 per cent. of the total phosphorus, this being a decrease of about 4 per cent. from the normal total phosphorus content. The increase in the water-soluble phosphorus has been more marked, the soluble phosphorus now constituting 47.8 per cent. of the total. In the normal spleen 32 per cent. of the total phosphorus was soluble. The increase is due to additions from the phosphatid and nuclein phosphorus. The decrease in the insoluble phosphorus was also more marked, the percentage dropping to 31.4 per cent. of the total phosphorus, the normal being 40.1 per cent. The water-soluble nitrogen was likewise markedly increased, being 26.5 per cent. of the total, the normal being 5.7 per cent., the increase in the soluble nitrogen being due mainly to the amino acids, peptones, and simpler nitrogen derivatives; while the insoluble nitrogen suffered a corresponding decrease, being 73.5 per cent. of the total nitrogen, the normal being 94.3 per cent.

PLATE 64.

FIGS. 5 and 6. Dog spleen after autolysis for fifty-three hours. Karyolysis has neared completion, only shadows of basophilic nuclear material being left. Of the nuclein nitrogen 27.4 per cent. has become soluble, while 72.6 per cent. is still insoluble. The phosphorus in the phosphatid form has decreased still further in amount, being now only 16 per cent. of the total phosphorus. Partly as a result of this and partly because of the addition of nuclein phosphorus, the water-soluble phosphorus increase is again evident, now amounting to 57.7 per cent. of the total phosphorus. The insoluble phosphorus has decreased to 22 per cent. of the total phosphorus. The water-soluble nitrogen has increased but slightly since the first thirty hours of autolysis, and is now 28.6 per cent. of the total. The insoluble nitrogen has likewise decreased at a slower rate, being now 71.4 per cent. of the total nitrogen. Apparently a point of slow change has now been reached.

PLATE 65.

FIGS. 7 and 8. Dog spleen after autolysis for six days (144 hours). Nuclear destruction is now complete as far as histological changes are concerned, and the other tissue changes have practically reached a standstill. Of the nuclein nitrogen 28 per cent. has become soluble, while 72 per cent. is still insoluble in

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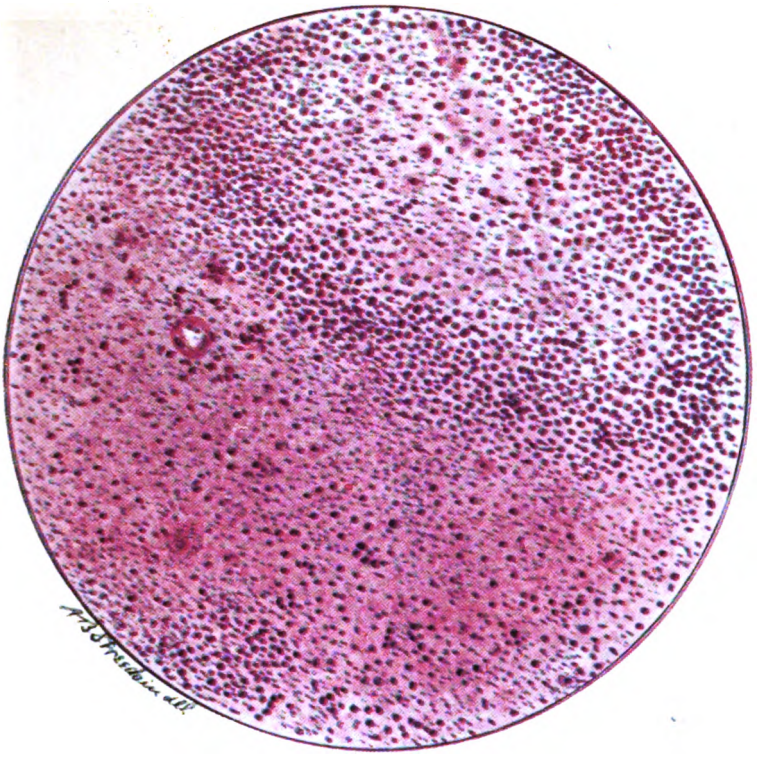


FIG. 1.

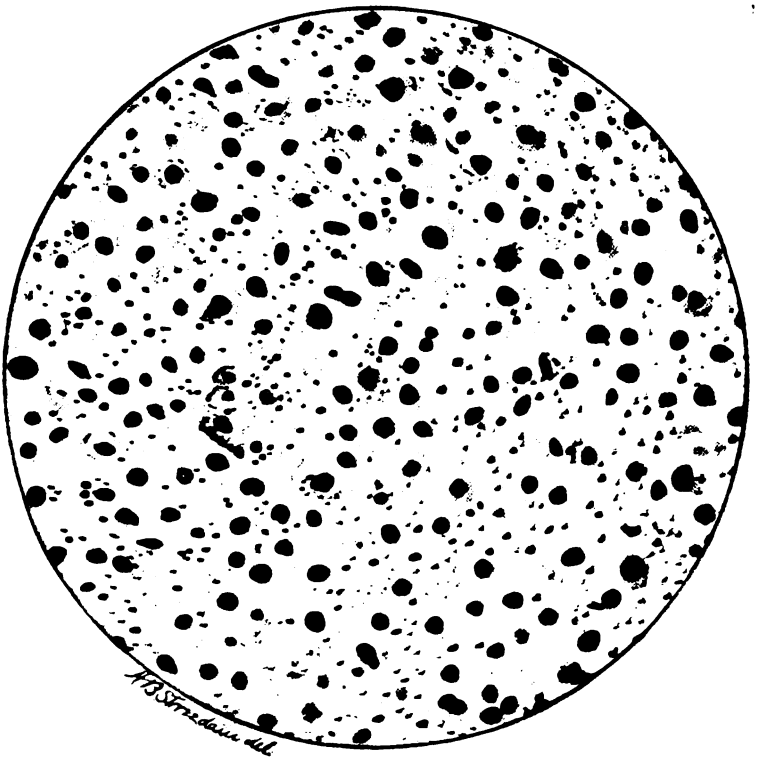


FIG. 2.

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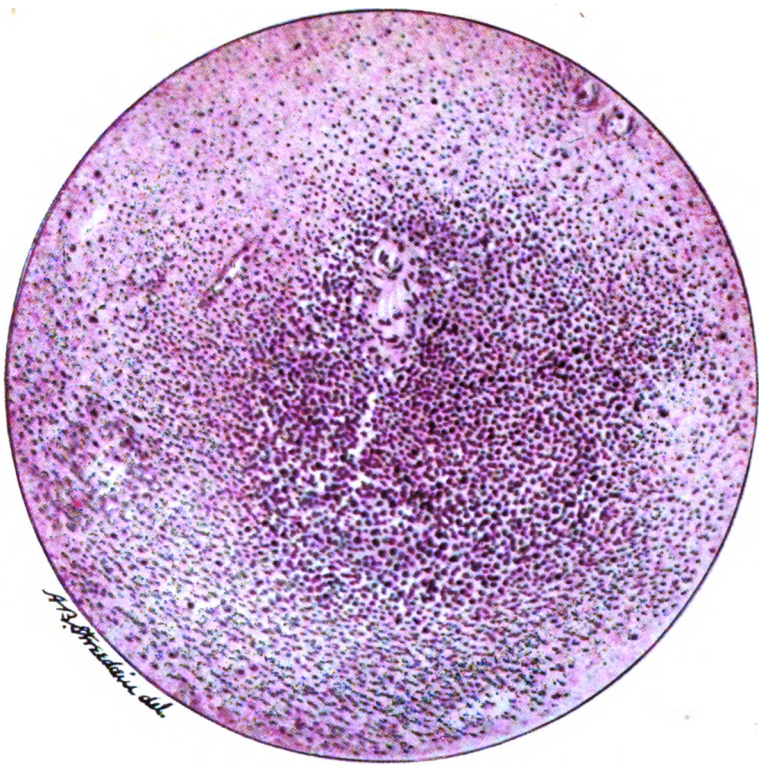


FIG. 3.

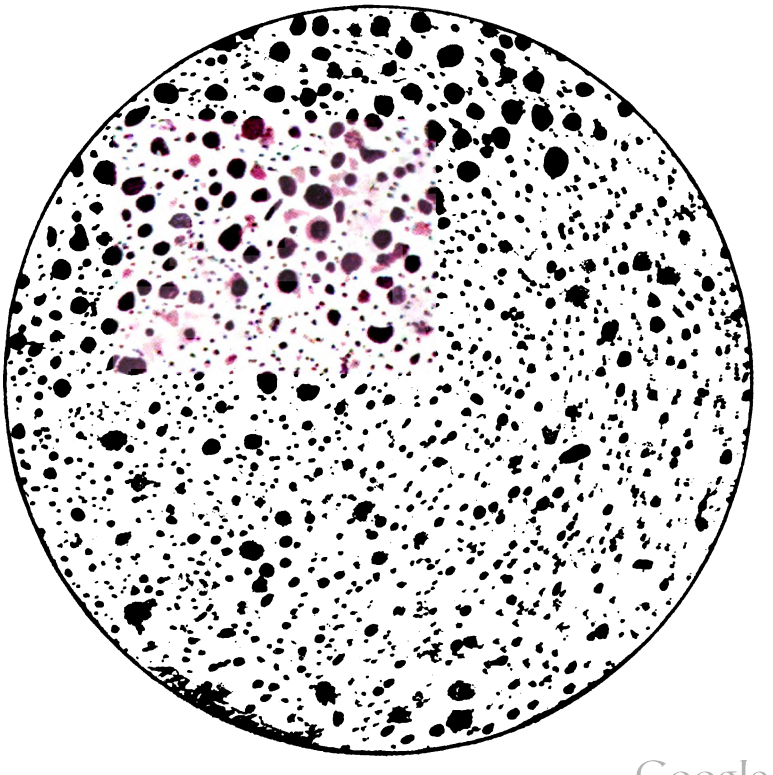


FIG. 4.

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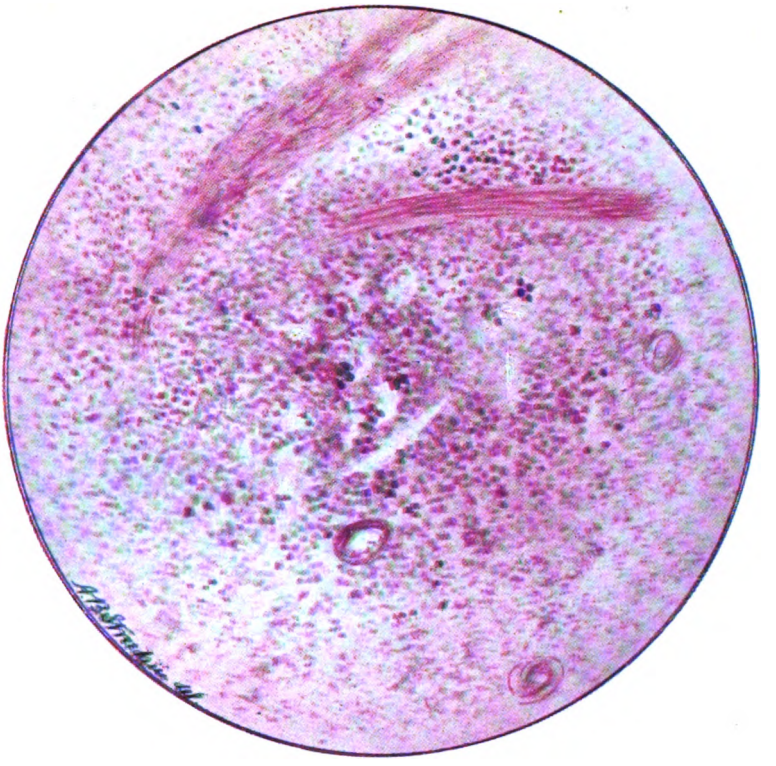


FIG. 5.

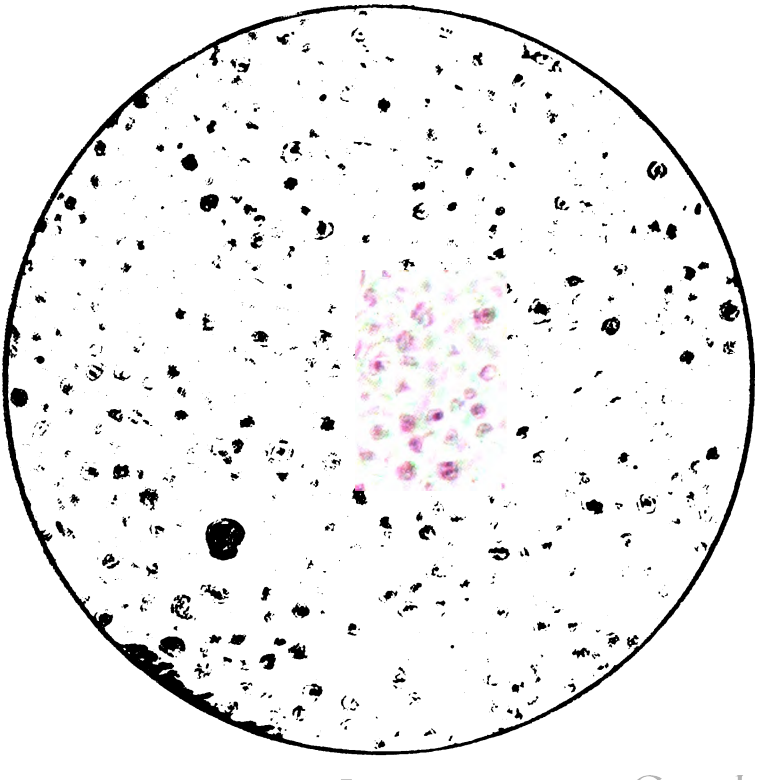


FIG. 6.

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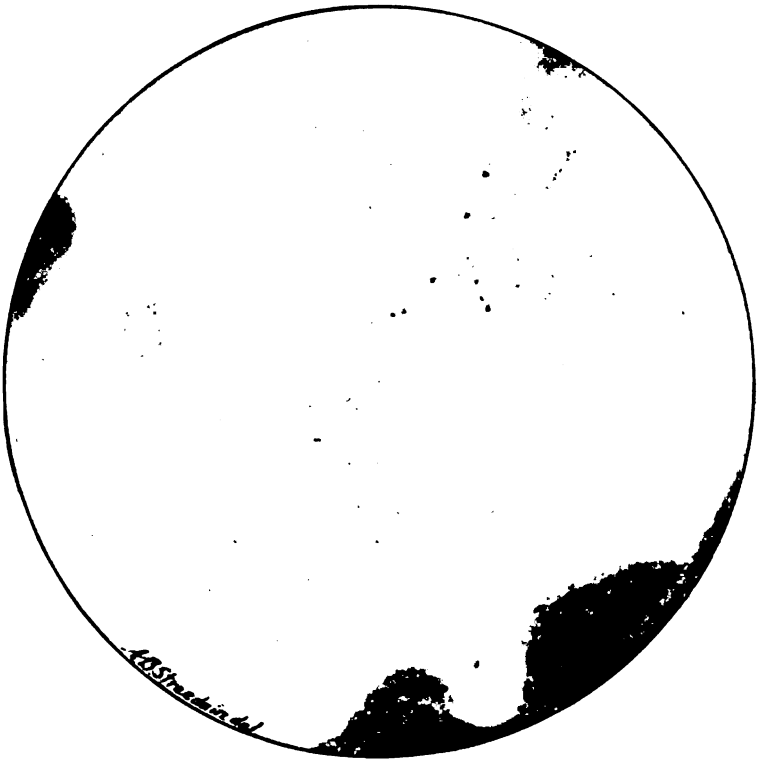


FIG. 7.

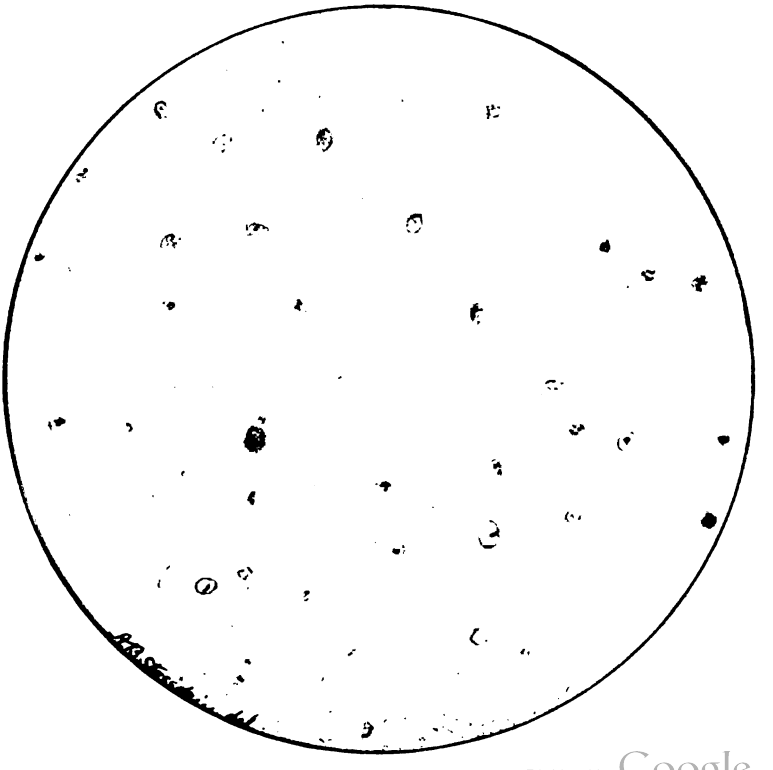


FIG. 8.

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spite of the loss of all visible nuclear structures. The phosphorus in the phosphatid form has decreased to about half the normal amount, leaving only about 13.6 per cent. of the total phosphorus in this form. The water-soluble phosphorus has increased, now being 67 per cent. of the total, or practically double the amount in the normal spleen. The insoluble phosphorus has decreased to 18.8 per cent. of the total phosphorus, this percentage being about half the normal content, which indicates that the phosphatid phosphorus is set free more rapidly than the nuclein phosphorus, only a slight increase in the water-soluble nitrogen having been noted after autolysis for fifty-three hours. The water-soluble nitrogen is now 30.3 per cent. of the total nitrogen, which is a fivefold increase over the normal. The insoluble nitrogen has slowly decreased, now being 69.7 per cent. of the total nitrogen, a decrease from the normal of about 30 per cent.

The microscopical examination of another dog spleen that had been autolyzed *in vivo* for two days resembled very closely spleen H (figures 7 and 8). Chemically, however, there were characteristic differences. Soluble nuclein nitrogen was absent and there was only 0.3 per cent. of nuclein nitrogen in the insoluble form. The phosphorus in the phosphatid form had decreased markedly in amount, only about 4 per cent. of the total phosphorus being left, and this total had markedly decreased. The water-soluble phosphorus was about the same, or a little more than normally, being 38.86 per cent. of the total phosphorus still present, but compared with the normal spleen content the water-soluble phosphorus had actually decreased one fourth in amount. The insoluble phosphorus constituted a greater percentage than normally of the total phosphorus still present, being 54 per cent., although it had actually decreased to about one third or one fourth of the amount present in the normal spleen. The percentage of the water-soluble nitrogen was about normal, being 5.7 per cent. The percentage was low because the soluble nitrogen was removed as rapidly as it was formed. Of the entire nitrogen of this spleen 94.3 per cent. was still insoluble.

The rate of autolysis *in vivo* was thus shown to be much more rapid than that *in vitro* at room temperature, but the diffusion of the soluble products of autolysis prevented their accumulation in the necrotic spleen tissue *in vivo*. Moreover, the disintegration of nucleins was much more extensive in the autolysis *in vivo* than in that *in vitro*, about six sevenths of the nuclein nitrogen having disappeared.

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COMPLETE BALANCE STUDIES OF NITROGEN, SULPHUR, PHOSPHORUS, CALCIUM, AND MAGNESIUM IN INTESTINAL INFANTILISM

By F. H. McCRUDDEN AND HELEN L. FALES

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COMPLETE BALANCE STUDIES OF NITROGEN, SULPHUR, PHOSPHORUS, CALCIUM, AND MAGNESIUM IN INTESTINAL INFANTILISM.*

BY F. H. McCRUDDEN AND HELEN L. FALES.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

PLATES 66–68.

Certain preliminary investigations, published[1] and unpublished, suggested that there was probably a disturbance of the proper balance between the intake and output of material in the cases to which Herter[2] has applied the name intestinal infantilism. To investigate this possibility, complete balance observations were carried out in which the total intake in the food and output in the urine and feces of nitrogen, sulphur, phosphorus, calcium, and magnesium for periods of six days were determined. These observations were upon the two cases of infantilism described in previous papers. As a control on these two cases, a normal child was similarly studied. The diet was a generous, unrestricted one, only non-homogeneous foods being excluded. All the food was weighed and analyzed as usual. The technique employed in carrying on these investigations was essentially that described in detail in earlier papers by one of us.[3]

The results are given in tables I, II, and III. The methods of analysis and the amounts used for analysis are shown in table IV.

Considerations similar to those in an earlier paper[4] indicate that the probable errors in the figures for the balances are within the following limits: Nitrogen, ± 0.2 of a gram; sulphur, ± 0.05 of a

* Received for publication, February 24, 1912.

[1] McCrudden, *Jour. Exper. Med.*, 1912, xv, 107; McCrudden and Fales, *idem*, 113.

[2] Herter, On Infantilism from Chronic Intestinal Infection, New York, 1908.

[3] McCrudden, *Am. Jour. Physiol.*, 1905, xiv, 389; 1906–7, xvii, 211.

[4] McCrudden, *Arch. Int. Med.*, 1912, ix, 273.

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TABLE I.

Boy, F. S., with Infantilism. Weight at Beginning of Experiment, 14.1 Kilograms; Weight at End, 14.5 Kilograms.

	Day.	Nitrogen.	Calcium oxid.	Magnesium oxid.	Phosphorus pentoxid.
Urine	1	2.75	0.010	0.005	0.512
	2	2.76	0.016	0.070	0.520
	3	3.02	0.021	0.086	0.572
	4	2.75	0.012	0.074	0.497
	5	2.51	0.009	0.054	0.398
	6	2.32	0.040	0.111	0.211
	Total	16.11	0.108	0.400	2.710
	Total weight.				
Feces	1.....29.6	1.707	1.965	0.213	0.855
	2.....14.32	0.868	0.750	0.009	0.767
	3.....58.5	3.532	2.744	0.579	2.495
	4.....40.35	2.840	1.733	0.339	1.077
	5.....52.55	2.286	1.618	0.331	1.072
	6.....35.75	2.288	1.451	0.229	1.073
	Total.....231.1	13.52	10.26	1.700	7.34
Total excretion		29.63	10.37	2.10	10.25
Total in food		35.64	6.35	2.43	11.91
Retention		6.01	....	0.33	1.66
Loss		....	4.02	....	....
Per cent. retained		16.9	....	1.4	13.9
Per cent. lost		....	63.3	....	....

gram; calcium oxid, ± 0.04 of a gram; magnesium oxid, ± 0.02 of a gram; phosphorus pentoxid, ± 0.07 of a gram.

Calcium.—Examination of the balances showed a striking loss of calcium by F. S., in spite of the fact that everything else was retained. In the case of F. H., magnesium as well as calcium was lost. Sulphur and nitrogen were retained. The results in these two cases were in striking contrast to those of the normal boy in which all the elements were retained in proportion to the amounts in which they were metabolized.

In the two cases of infantilism, the calcium excretion in the urine was almost negligible, the amount excreted in a week being less than the amount normally excreted in a day. The amount of calcium in the urine was only 1 to 2 per cent. of the amount in the feces, this being in marked contrast to the normal case where it was 20 per cent. Practically all the calcium was in the feces. In the

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TABLE II.

Boy, F. H., with Infantilism. Weight at Beginning of Experiment, 11.8 Kilograms; Weight at End, 12.2 Kilograms.

Day.		Nitrogen.	Sulphur.	Calcium oxid.	Magnesium oxid.
Urine	1	5.68	0.432	0.050	0.061
	2	5.44	0.412	0.017	0.058
	3	5.27	0.392	0.014	0.063
	4	5.62	0.428	0.028	0.068
	5	5.87	0.452	0.018	0.073
	6	5.56	0.424	0.029	0.067
	Total	33.44	2.540	0.156	0.390
	Total weight.				
Feces	1 ... 17.4	0.789	0.089	1.583	0.300
	2 ... 21.3	0.989	0.117	1.632	0.337
	3 ... 17.5	0.836	0.099	1.279	0.287
	4 ... 18.7	0.899	0.102	1.265	0.308
	5 ... 22.7	1.016	0.154	1.577	0.336
	6 ... 19.7	0.875	0.114	1.449	0.274
	Total ... 117.3	5.404	0.675	8.785	1.842
Total excretion		38.84	3.216	8.941	2.231
Total in food		48.77	4.110	8.820	1.968
Retention		9.93	0.894	....	....
Loss		....	....	0.121	0.263
Per cent. retained		20.3	21.8	....	....
Per cent. lost		....	....	1.4	1.3

case of F. H., there was as much calcium in the feces as in the food, and in the case of F. S., 60 per cent. more than in the food.

Magnesium.—The magnesium, like the calcium, was low in the urine and high in the feces. In the urine of the normal boy, the quantity of magnesium was 56 per cent. of that in his feces, while in the urine of the two patients with infantilism, it was only about 20 per cent. of that in the feces. The feces of the normal boy contained about half as much magnesium as was present in his food; whereas the feces of F. S. contained more than two thirds as much, and the quantity in the feces of F. H. nearly equalled that in his food.

Nitrogen.—In the stools the amount of nitrogen was high. In F. S. it was over two grams per day. The absolute amount in F. H. was not greater than in the normal boy, but it was nearly twice as great in proportion, since he was receiving only about half as much per day in his food. If we compare the amount in the feces with

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TABLE III.

Normal boy, W. McC. Weight at Beginning of Experiment, 27 Kilograms; Weight at End, 27.2 Kilograms.

Day.		Nitrogen.	Sulphur.	Calcium oxid	Magnesium oxid.	Phosphorus pentoxid.
Urine	1.....	10.43	0.729	0.374	0.153	2.34
	2.....	10.65	0.744	0.406	0.139	2.22
	3.....	10.35	0.717	0.373	0.154	2.23
	4.....	11.07	0.832	0.348	0.152	2.06
	5.....	11.35	0.808	0.332	0.157	2.58
	6.....	11.12	0.810	0.390	0.154	2.68
Total...		64.97	4.640	2.223	0.909	14.11
Feces	Dry feces					
	1 ...22.8	1.232	0.133	1.518	0.267	0.686
	2 ...19.0	0.997	0.085	1.846	0.263	0.718
	3 ...20.9	1.165	0.115	1.784	0.288	0.768
	4 ...23.5	1.173	0.113	2.084	0.301	0.839
	5 ...15.3	0.811	0.063	1.318	0.220	0.526
	6 ...21.1	1.110	0.073	1.643	0.279	0.856
Total 122.6		6.488	0.582	10.193	1.618	4.393
Total excretion .		71.46	5.222	12.42	2.53	18.50
Total in food ...		86.10	6.138	14.988	3.263	24.709
Retention.....		14.64	0.916	2.57	0.73	6.20
Per cent. retained.....		17.0	14.9	17.1	22.4	25.0

TABLE IV.

		Nitrogen.	Calcium oxid and magnesium oxid.	Sulphur.	Phosphorus pentoxid.
Amounts used for analysis	urine.....	5 c.c.	200 c.c.	50 c.c.	50 c.c.
	food.....	1.0000 gm.	10.0000 gm.	2.0000 gm.	2.0000 gm.
	feces.....	1.0000 gm.	1.0000 gm.	2.0000 gm.	1.0000 gm.
Method of analysis.....		Kjeldahl	McCrudden[5]	Folin[6]	Neumann[7]

that in the food, we see that it was 38 per cent. in the case of F. S., 11 per cent. in the case of F. H., and only 7.5 per cent. in the case of the normal boy.

In the urine, the fraction of the nitrogen of the food that appeared was smaller than normally, being 45 per cent. in the case of F. S., 67 per cent. in the case of F. H., and 91 per cent. in the case of the normal boy.

[5] McCrudden, *Jour. Biol. Chem.*, 1911, x, 187.

[6] Folin, *Jour. Biol. Chem.*, 1905–6, i, 131.

[7] Neumann, *Ztschr. f. physiol. Chem.*, 1902, xxxvii, 131; Neumann and Meinertz, *idem*, 1904–5, xliii, 37.

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The amount of nitrogen in the feces was brought out particularly well by the high ratio which the nitrogen of the feces bore to that of the urine. This was 84:100 in the case of F. S., 16:100 in the case of F. H., and only 10:100 in the case of the normal boy.

Sulphur.—The figures for sulphur, which, like nitrogen, is a measure of protein metabolism, show changes similar to those for nitrogen. The sulphur was not determined in the case of F. S., but in that of F. H. the sulphur excretion in the feces was more marked than that of the nitrogen. The figures are as follows:

	Sulphur proportion: feces to food.	Sulphur proportion: urine to food.	Sulphur proportion: feces to urine.
F. H.	16.5	62.0	27.0
Normal boy	9.5	76.0	12.5

Furthermore, even the absolute amount of sulphur in the feces in F. H. was greater than in the case of the normal boy, in spite of the fact that F. H. was much smaller and had less sulphur in his food.

Phosphorus.—The excretion of phosphates in the stools in infantilism was larger and more marked even than that of calcium, magnesium, nitrogen, and sulphur had been. In the feces of F. S., there was nearly three times as much phosphate as in his urine, while in the urine of the normal boy, there was over three times as much phosphate as in his stools. In the feces of the normal boy, there was only about one sixth as much phosphate as in his food, while in the feces of F. S. there was over three fifths as much. There was nearly double as much phosphate in the feces of F. S. as in the feces of the normal boy, although the latter had over twice as much phosphorus in his food.

The daily weight of the dry feces was large, being especially so in the case of F. S., where it averaged 3.9 grams per day. The weight of the feces of F. H. was also large in proportion to his size and the amount of food he took.

DISCUSSION.

To summarize briefly, the quantities of nitrogen, sulphur, phosphates, calcium, and magnesium in the feces were high, and the quantities in the urine were low. As an explanation of this condi-

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tion, we might think of decreased absorption or increased excretion. If the disturbance affected only the calcium, we might consider increased excretion as a possible cause, but the other elements, especially the sulphur and nitrogen, are not generally excreted in any considerable quantity into the intestines, so it seems probable that lack of absorption must be responsible for the large amounts of these elements.

The disturbance of the calcium metabolism was more marked than that of the other elements. In the case of F. S., the ratio of the calcium to the phosphates in the urine was 4:100, and in the normal boy it was 16:100. The ratio of calcium to magnesium in the urine was 27:100 in the case of F. S., 40:100 in the case of F. H., and about 244:100 in the case of the normal boy. The fact that the excretion of calcium was considerably greater than normally in the two cases of infantilism, when taken in connection with the fact that in one of the cases there was actually a greater amount of calcium in the feces than in the food, must mean that in the case of calcium, in addition to the lack of absorption, there was also an increased excretion through the intestines.

The great loss of calcium may account for the fragility of the bones that was noted, for this led in the case of F. S. to two fractures from very slight causes, these fractures occurring within a few months of each other. X-ray examinations of the skeletal system showed disturbances in accord with these results. The epiphyses throughout were ununited, some of the smaller bones, especially those of the wrist, were missing or uncalcified, and the cortex of the long bones was found to be exceedingly thin, both in absolute figures and in comparison with the diameter of the bone. Examples of these conditions are seen in figures 1 and 2. In figure 1, the right wrist of F. S. is shown, while in figure 2 the bones of the lower part of the leg are seen. By way of comparison, in figure 3 are pictured the leg bones of a frail, eight year old girl of the same weight as F. S., who is an eleven year old boy. There is, in fact, a condition of osteoporosis.

These results are in accord with the facts already pointed out by one of us in various papers on the subject of osteomalacia; namely that this condition should be regarded, not as a distinct disease, but

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as a symptom following excessive calcium catabolism, which may be brought about by a variety of different conditions.[8]

The results of the present paper have suggested a number of interesting points which are at present under investigation and are, for this reason, reserved for future papers.

SUMMARY.

In two cases of infantilism there was poor absorption from the intestines of nitrogen, sulphur, phosphorus, calcium, and magnesium.

The excretion of these elements in the urine was low. The excretion of calcium in the urine was so small as to be negligible.

The excretion of calcium into the intestines was increased.

The complete balance experiments showed that while other elements were retained, calcium was lost.

EXPLANATION OF PLATES.

PLATE 66.

FIG. 1. X-ray photograph of the hands of F. H. (infantilism). Some of the carpal bones are missing or uncalcified. The cortex of the bones is seen to be very thin.

PLATE 67.

FIG. 2. X-ray photograph of a leg of F. H. (infantilism). The cortex of the bones is seen to be very thin compared with that of a normal child (figure 3).

PLATE 68.

FIG. 3. X-ray photograph of the legs of a normal child, 9 years old. For comparison with figure 2.

[8] McCrudden, *Arch. Int. Med.*, 1910, v, 596; 1912, ix, 273.

Further evidence on this point is the statement by Trevelyan (Albutt and Rolleston, System of Medicine, 1908, iv, pt. 1, 390) that a condition of osteoporosis is sometimes observed accompanying the increased bone formation in acromegaly.

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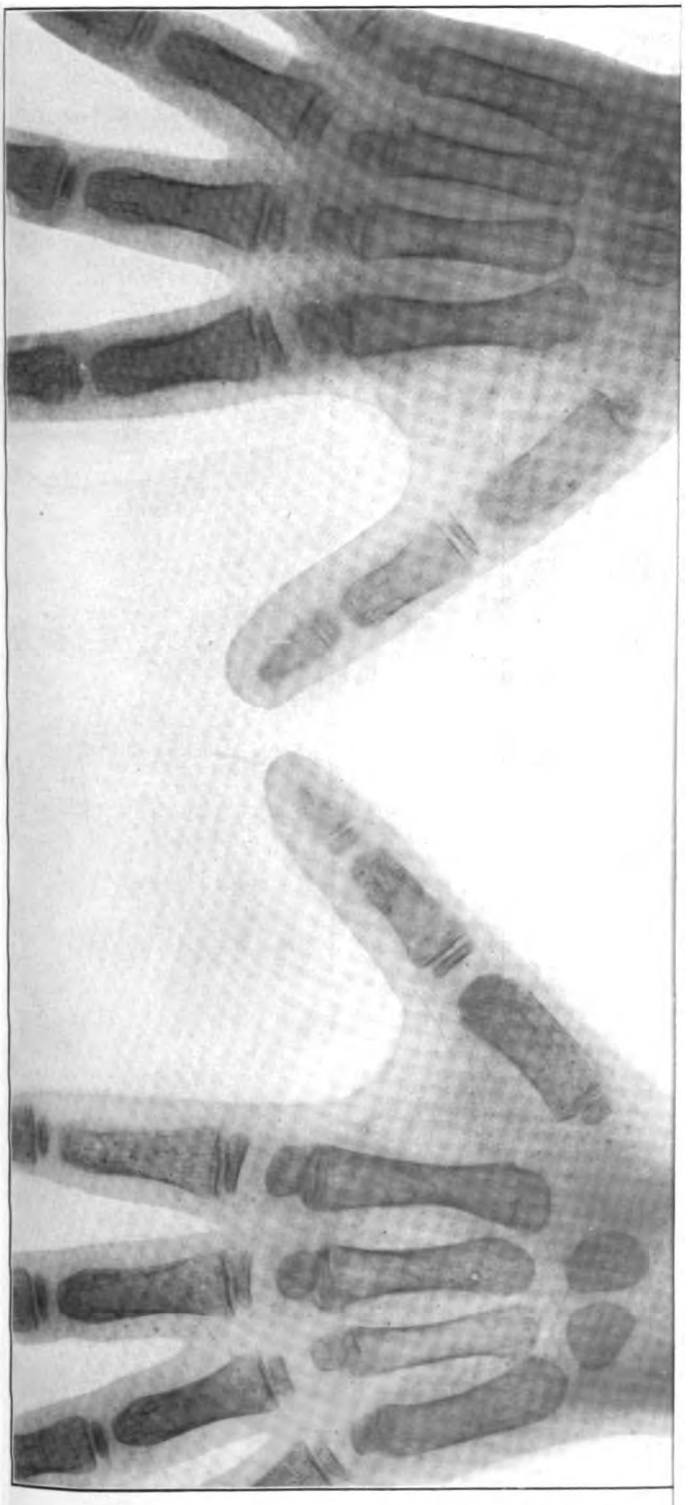


FIG. 1.

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FIG. 2.

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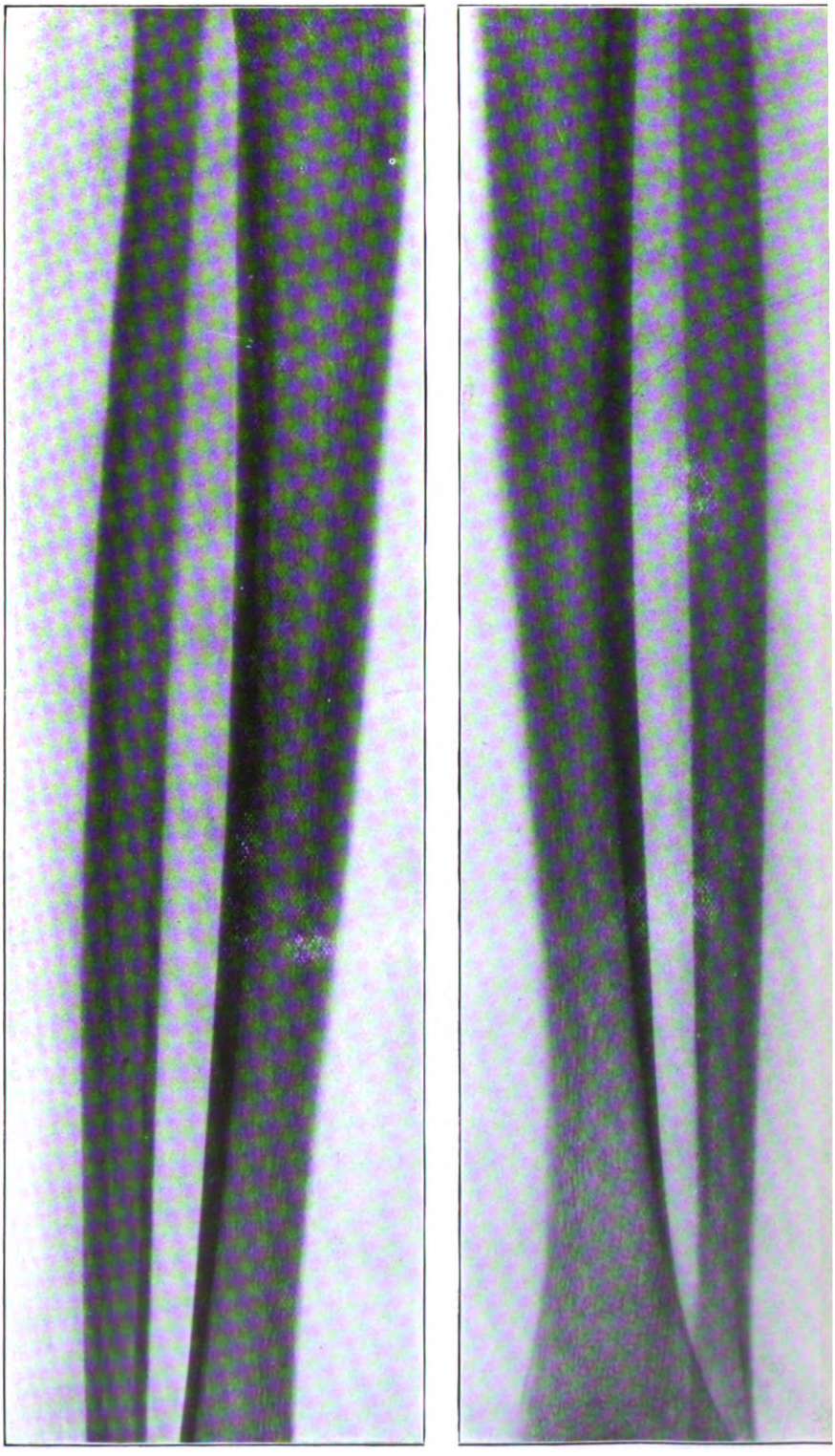


FIG. 3.

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THE EFFECT OF FAT AND OF CARBOHYDRATE DIETS ON THE EXCRETION OF CREATIN IN CASES OF RETARDED DEVELOPMENT.*

BY FRANCIS H. McCRUDDEN.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

Creatin is not a normal constituent of human urine. It occurs in the urine during starvation and inanition, and Cathcart[1] has shown that administration of carbohydrates leads to its disappearance. The disappearance of creatin from the urine of starving rabbits may be brought about in the same way.[2] Likewise in the diabetes in dogs caused by phloridzin, the feeding of carbohydrates leads to the disappearance of creatin.[3] Only carbohydrates have this effect. Fat does not reduce the creatin excretion.

It was observed by the writer that creatin was constantly excreted in certain cases of retarded development, and preliminary observations seemed to indicate that the amount of creatin excreted was greater on a diet rich in carbohydrates than on a diet rich in fats.[4] In view of the great interest and diversity of opinion regarding the metabolism of creatin and creatinin at the present time, and in view also of the bearing which the results might have in the clearing up of the nature of the process in cases of retarded development, the unexpected results of my preliminary observations, showing that on a high carbohydrate diet more creatin may be excreted than on a high fat diet, seemed worthy of further study. The result of comparing the effect of these two diets upon the excretion of creatin in cases of retarded development is given in this paper.

Four patients were used for the observations. Two of them,

* Received for publication, February 24, 1912.

[1] Cathcart, *Jour. Physiol.*, 1909, xxxix, 311.

[2] Mendel and Rose, *Jour. Biol. Chem.*, 1911, x, 213.

[3] Cathcart and Taylor, *Jour. Physiol.*, 1910, xli, 276.

[4] McCrudden, *Jour. Exper. Med.*, 1912, xv, 107.

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F. S. and J. P., have been referred to in earlier papers,[5] E. B. was an achondroplasiac dwarf, and M. S. a cretin. The observations on each patient were divided into three periods of four days each: first, a preliminary period during which the food consisted of the usual mixed diet of the hospital; second, a period in which the diet was rich in fats; and third, a period in which the diet was rich in carbohydrates.

In the second period, as large amounts as possible of cream, olive oil, and butter were given; the meat, consisting of young chicken, ham, pork, and bacon, was selected to contain as much fat as possible; and the bread, potatoes, and sugar were reduced to the lowest quantities. The bread was cut thin and was thickly smeared with butter, and the potatoes were mashed and contained as much moisture and butter as possible.

In the third, or carbohydrate period, as much starchy food as possible was given. No butter was used, and instead of cream, separated milk, *i. e.*, milk from which the fat had been almost completely removed, was given. Moreover, the meat selected was as lean as possible. In the diets given below the solid foodstuffs are reported in grams, the liquids in cubic centimeters. The results follow:

Patient I.—F. S., boy, 11 years old. Diagnosis: infantilism of the type of Herter. Height, 106.6 cm. Weight at the beginning of the experiment, 22.1 kilos; weight at the end of the experiment, 22.3 kilos.

I. *Preliminary Period.*—1st to 4th day.

II. *Fat-rich Period. 5th day. Breakfast.*—Bread, 10; cocoa, 125; bacon, 39. *Dinner.*[6]—Cream, 16; bread, 12; sweet potato, 70; mutton chop, 85; olive oil, 30. *Supper.*—Bread, 12; bacon, 37; cream, 40; orange, 92; cocoa, 120.

6th day. Breakfast.—Apple, 57; toast, 15; cream, 45; cocoa, 110; lamb chop, 85; olive oil, 30. *Dinner.*—Bread, 10; bacon, 42; lamb chop, 52; olive oil, 30. *Supper.*—Bread, 12; orange, 72; cream, 50; cocoa, 110; ham, 85; olive oil, 30.

7th day. Breakfast.—Apple, 60; toast, 10; cream, 50; cocoa, 110; bacon, 35; olive oil, 30. *Dinner.*—Bread, 10; orange, 10; cream, 75; mutton chop, 50; olive oil, 30. *Supper.*—Bread, 9; baked apple, 47; cream, 75; cocoa, 110.

8th day. Breakfast.—Apple, 35; toast, 9; cream, 60; cocoa, 110; lamb chop, 47; olive oil, 30. *Dinner.*—Sweet potato, 35; bread, 9; cream, 50; cocoa, 110; lamb chop, 60. *Supper.*—Bread, 9; cream, 65; cocoa, 110; apple, 50; ham, 62.

In addition, during these four days a total of 136 grams of butter and 130 grams of sugar was given.

[5] McCrudden, *loc. cit.;* McCrudden and Fales, *Jour. Exper. Med.*, 1912, xv, 113; McCrudden and Fales, *idem*, 450.

[6] Through an oversight, some banana was given at this meal.

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III. *Carbohydrate-rich Period. 9th day. Breakfast.*—Grape fruit, 116; toast, 29; separated milk, 150. *Dinner.*—Rice, 52; beef, 49; beans, 19; biscuit, 11; bread, 18; tapioca and fruit, 60. *Supper.*—Bread, 29; honey, 5; separated milk, 150; meat, 60.

10th day. Breakfast.—Toast, 19; marmalade, 20; grape fruit, 89; separated milk, 150. *Dinner.*—Rice, 73; bread, 24; gelatin, 122; honey, 16; beef, 37. *Supper.*—Bread, 25; jam, 22; separated milk, 150; meat, 89.

11th day. Breakfast.—Grape fruit, 94; toast, 20; marmalade, 22; beefsteak, 67. *Dinner.*—Bread, 20; honey, 15; rice, 75; tapioca and fruit, 115; beefsteak, 92. *Supper.*—Bread, 28; apple, 83; jam, 20; biscuit, 9; meat, 85; separated milk, 85.

12th day. Breakfast.—Orange, 142; toast, 22; marmalade, 20; separated milk, 150. *Dinner.*—Bread, 17; rice, 83; gelatin, 109; honey, 10; steak, 87. *Supper.*—Bread, 19; meat, 65; jam, 20; separated milk, 200.

In addition, a total of 33 grams of sugar was given during this period.

Patient II.—J. P., boy, 17 years old. Diagnosis: infantilism of the type of Herter. Height, 118.2 cm. Weight at the beginning of the experiment, 22.2 kilos; weight at the end, 22.9 kilos.

I. *Preliminary Period.*—1st to 4th day.

II. *Fat-rich Period. 5th day. Breakfast.*—Bread, 10; egg, 120. *Dinner.*[1]—Cream, 16; bread, 12; potato, 70; mutton chop, 82; olive oil, 30. *Supper.*—Bread, 12; cream, 50; sardines, 7; orange, 85; cocoa, 120.

6th day. Breakfast.—Apple, 44; egg, 90; toast, 15; cream, 75; cocoa, 100; olive oil, 30. *Dinner.*—Lamb chop, 38; potato, 40; bread, 10; olive oil, 30. *Supper.*—Bread, 12; orange, 72; onion, 10; salmon, 20; olive oil, 30.

7th day. Breakfast.—Apple, 50; toast, 10; cream, 50; cocoa, 110; egg, 92; lamb chop, 18; olive oil, 30. *Dinner.*—Potato, 18; bread, 10; orange, 10; cream, 75; chicken, 73; olive oil, 30. *Supper.*—Bread, 9; egg, 45; cream, 75; cocoa, 110; baked apple, 47.

8th day. Breakfast.—Apple, 35; toast, 9; cream, 60; cocoa, 110; egg, 25; olive oil, 15. *Dinner.*—Sweet potato, 35; bread, 9; cream, 50; cocoa, 110; lamb chop, 60. *Supper.*—Bread, 9; apple, 30; cream, 65; cocoa, 110; lamb chop, 34; olive oil, 30.

In addition, a total of 215 grams of butter and 96 grams of sugar was given during this period.

III. *Carbohydrate-rich Period. 9th day. Breakfast.*—Orange, 90; toast, 29; separated milk, 150. *Dinner.*—Bread, 18; beans, 39; potato, 49; chicken, 38; biscuit, 9; separated milk, 150. *Supper.*—Bread, 38; tomato, 42; honey, 5; separated milk, 150; scraped beef, 60.

10th day. Breakfast.—Toast, 28; marmalade, 6; orange, 71; separated milk, 150. *Dinner.*—Bread, 22; potato, 50; honey, 18; gelatin, 122; chicken, 60; gravy, 15. *Supper.*—Bread, 25; jam, 17; separated milk, 150; meat, 80.

11th day. Breakfast.—Apple, 60; oatmeal, 100; separated milk, 150; toast, 7; steak, 35. *Dinner.*—Chicken, 42; steak, 32; bread, 19; separated milk, 150; tapioca and fruit, 100. *Supper.*—Bread, 27; jam, 20; apple sauce, 83; biscuit, 9; meat, 97; separated milk, 150.

[1] Through an oversight, some banana was given at this meal.

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12th day. Breakfast.—Orange, 132; toast, 26; honey, 15; separated milk, 150. *Dinner.*—Bread, 3; onion, 8; chicken, 12; potato, 19; gelatin, 50; bread, 20; honey, 11. *Supper.*—Bread, 37; steak, 110; tomato, 55; jam, 18; separated milk, 250.

In addition, a total of 65 grams of sugar was given during this period.

Patient III.—E. B., boy, 4 years old. Diagnosis: achondroplasia. Height, 78 cm. Weight at the beginning of the experiment, 14.8 kilos; weight at the end, 14.5 kilos.

I. *Preliminary Period.*—1st to 4th day.

II. *Fat-rich Period. 5th day. Breakfast.*—Baked apple, 70; egg, 82; bread, 8; cream, 100; cocoa, 70. *Dinner.*—Chicken, 54; potato, 30; bread, 8; cream, 60. *Supper.*—Biscuit, 8; grapes, 25; egg, 70; bread, 8; cream, 75; cocoa, 70.

6th day. Breakfast.—Orange juice, 100; bacon, 20; egg, 75; toast, 9; cream, 75; cocoa, 70. *Dinner.*—Bread, 9; chicken, 70; potato, 25; jelly, 20; cream, —. *Supper.*—Bread, 8; cream, 75; cocoa, 70; egg, 57.

7th day. Breakfast.—Egg, 85; toast, 8; orange juice, 85; cream, 65; cocoa, 70. *Dinner.*—Bread, 7; pork, 62; potato, 20; water, 200. *Supper.*—Bread, 8; pork, 100; cream, 65; cocoa, 90; apple, 50.

8th day. Breakfast.—Bacon, 15; egg, 50; cream, 75; cocoa, 70; toast, 9; orange juice, 100. *Dinner.*—Bread, 8; potato, 20; chicken, 35. *Supper.*—Bread, 8; egg, 85; biscuit, 5; cream, 95; cocoa, 110.

In addition, during the whole period a total of 113 grams of sugar and 118 grams of butter was used.

III. *Carbohydrate-rich Period. 9th day. Breakfast.*—Toast, 27; honey, 8; oatmeal, 100. *Dinner.*—Meat, 50; apple, 100; potato, 50. *Supper.*—Bread, 12; meat, 50; beans, 70; honey, 10; biscuit, 7.

10th day. Breakfast.—Cereal, 100; orange juice, 110; toast, 14; separated milk, 150. *Dinner.*—Bread, 15; potato, 70; scraped beef, 50; jelly, 12. *Supper.*—Meat, 50; bread, 11; apple, 54; separated milk, 150; biscuit, 22.

11th day. Breakfast.—Toast, 23; orange, 120; honey, 8; scraped beef, 45; separated milk, 150. *Dinner.*—Beans, 50; potato, 50; meat, 50; water, 200. *Supper.*—Bread, 17; jelly, 8; apple sauce, 50; potato, 50; roast beef, 29; separated milk, 200.

12th day. Breakfast.—Orange, 120; oatmeal, 170; toast, 10; honey, 5; separated milk, 50. *Dinner.*—Potato, 40; meat, 50; rice, 59; water, 200. *Supper.*—Scraped beef, 62; bread, 15; fruit, 67; separated milk, 150.

In addition, 132 grams of sugar were used during this period.

Patient IV.—M. S., girl, 8 years old. Diagnosis: cretinism. Height, 90 cm. Weight at the beginning of the experiment, 16.9 kilos; weight at the end, 17.3 kilos.

I. *Preliminary Period.*—1st to 4th day.

II. *Fat-rich Period. 5th day. Breakfast.*—Bacon, 7; egg, 55; toast, 7; orange juice, 85; cream, 50. *Dinner.*—Bacon, 16; potato, 16; bread, 4; apple, 50; cream, 50. *Supper.*—Egg, 50; cream, 50; bread, 7; apple sauce, 35.

6th day. Breakfast.—Bacon, 21; toast, 4; egg, 40; cream, 70; orange juice,

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100. *Dinner.*—Chicken, 45; potato, 30; apple sauce, 39; cream, 50. *Supper.*—Egg, 40; bread, 5; cream, 75; prunes, 25.

7th day. Breakfast.—Orange juice, 110; egg, 40; cream, 80; toast, 6. *Dinner.*—Lamb chop, 53; bread, 5; apple sauce, 40; cream, 55. *Supper.*—Bread, 6; egg, 35; cream, 80.

8th day. Breakfast.—Orange juice, 110; egg, 30; bacon, 38; bread, 3; cream. 60. *Dinner.*—Pork, 55; apple sauce, 50; bread, 4; potato, 25. *Supper.*—Egg, 40, cream, 75; bread, 4.

In addition, a total of 27 grams of sugar and 125 grams of butter was used during this period.

III. *Carbohydrate-rich Period. 9th day. Breakfast.*—Cereal, 100; jelly, 25; bread, 15; separated milk, 130. *Dinner.*—Potato, 50; scraped beef, 50; soup, 130; bread, 15. *Supper.*—Bread, 14; jelly, 15; gelatin, 40; separated milk, 130.

10th day. Breakfast.—Oatmeal, 80; separated milk, 150; toast, 17; jelly, 12. *Dinner.*—Rice, 28; beans, 42; scraped beef, 41. *Supper.*—Bread, 20; strawberry jam, 30; separated milk, 150.

11th day. Breakfast.—Orange juice, 100; hominy, 126; separated milk, 150; toast, 12; jelly, 10. *Dinner.*—Tomato, 63; bread, 22; bean soup, 150; meat, 42. *Supper.*—Bread, 22; jam, 23; separated milk, 150.

12th day. Breakfast.—Cream of wheat, 92; separated milk, 155; toast, 22; strawberry jam, 12. *Dinner.*—Bean soup, 150; roast beef, 17; spinach, 60; potato, 62; bread, 19. *Supper.*—Bread, 22; separated milk, 250; apple sauce, 72.

13th day. Breakfast.—Apple, 52; oatmeal, 96; separated milk, 150; jelly, 20; toast, 15. *Dinner.*—Chicken, 43; potato, 50; bread, 22. *Supper.*—Bread, 28; separated milk, 210; jam, 10.

In addition, during this period there was given a total of 99 grams of sugar.

RESULTS.

TABLE I.

Patient, F. S.

Day.	Period.	Total nitrogen.	Creatinin plus creatin.	Creatinin.	Average creatinin.	Creatin.	Average creatin.
1	Preliminary	4.95	0.530	0.298	0.283	0.232	0.221
2		4.56	0.443	0.270		0.173	
3		4.34	0.455	0.312		0.143	
4		4.95	0.587	0.250		0.337	
5	Fat-rich diet	5.04	0.580	0.312	0.306	0.268	0.362
6		5.02	0.765	0.338		0.427	
7		4.03	0.572	0.253		0.319	
8		4.31	0.750	0.320		0.430	
9	Carbohydrate-rich diet	5.73	0.666	0.286	0.304	0.380	0.629
10		6.42	0.799	0.261		0.538	
11		9.94	1.312	0.328		0.984	
12		8.26	0.952	0.340		0.612	

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TABLE II.

Patient, J. P.

Day.	Period.	Total nitrogen.	Creatinin plus creatin.	Creatinin.	Average creatinin.	Creatin.	Average creatin.
1	Preliminary	5.34	0.502	0.354	0.375	0.148	0.226
2		5.48	0.545	0.373		0.172	
3		5.90	0.635	0.405		0.230	
4		6.76	0.723	0.368		0.355	
5	Fat-rich diet	5.77	0.567	0.354	0.326	0.213	0.229
6		4.77	0.536	0.289		0.247	
7		5.23	0.596	0.331		0.265	
8		3.61	0.521	0.329		0.192	
9	Carbohydrate-rich diet	5.05	0.723	0.405	0.319	0.318	0.491
10		6.43	0.800	0.415		0.385	
11		9.26	1.117	0.424		0.693	
12		8.42	0.999	0.432		0.567	

TABLE III.

Patient, E. B.

Day.	Period.	Total nitrogen.	Creatinin plus creatin.	Creatinin.	Average creatinin.	Creatin.	Average creatin.
1	Preliminary	5.68	0.444	0.222	0.224	0.222	0.411
2		3.62	0.389	0.214		0.175	
3		4.70	0.440	0.256		0.184	
4		3.40	0.370	0.203		0.167	
5	Fat-rich diet	4.06	0.381	0.202	0.189	0.179	0.342
6		5.26	0.381	0.202		0.179	
7		3.76	0.289	0.184		0.105	
8		3.85	0.316	0.167		0.149	
9	Carbohydrate-rich diet	4.76	0.491	0.263	0.239	0.228	0.618
10		5.61	0.611	0.212		0.399	
11		6.57	0.694	0.243		0.451	
12		6.49	0.677	0.237		0.440	

DISCUSSION.

The data show that the creatin excretion is higher on a carbohydrate-rich diet than on a fat-rich diet. The creatin excretion in the third period was increased over that in the second period by 74 per cent. in the case of F. S., by 115 per cent. in the case of J. P., by 81 per cent. in the case of E. B., and by 40 per cent. in the case of M. S. On the average, this was an increase of about 76 per cent. The creatin excretion in the case of M. S. was so variable from day to day that perhaps too much emphasis ought not to be laid on the

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TABLE IV.

Patient, M. S.

Day.	Period.	Total nitrogen.	Creatinin plus creatin.	Creatinin.	Average creatinin.	Creatin.	Average creatin.
1	Preliminary	3.41	0.378	0.322	0.339	0.057	0.058
2		3.83	0.455	0.370		0.085	
3		3.80	0.316	0.316		0.000	
4		4.29	0.437	0.347		0.090	
5	Fat-rich diet	3.24	0.312	0.312	0.294	0.000	0.055
6		3.70	0.390	0.312		0.078	
7		4.96	0.444	0.356		0.088	
8		3.18	0.277	0.217		0.060	
9[8]	Carbohydrate-rich diet	3.86	0.449	0.339	0.330	0.111	0.077
10		3.01	0.380	0.293		0.087	
11		3.77	0.382	0.332		0.051	
12		4.07	0.437	0.372		0.065	
13		3.99	0.398	0.315		0.082	

fact that its average was higher during the third period. At the same time, the presence of any creatin at all in the urine on a diet rich in carbohydrates is surprising. But in the other three cases, the increase was striking, the average excretion of creatin being about double during the carbohydrate-rich period.

The creatinin excretion was, on the whole, very constant and was not affected by the diet.

The results presented here are different from those heretofore obtained in studies of creatinuria, and show the inadequacy of the explanation that has previously been given for the appearance of creatin in the urine in conditions such as diabetes and starvation. According to this explanation, when the direct supplies of carbohydrate are cut off in these conditions, there is an increased catabolism of protein of the body tissues in order to form the necessary amounts of carbohydrate, and this abnormally increased catabolism of body protein is accompanied by the production of abnormal products, such as acetone and creatin. But, as we have seen, after the ingestion of larger amounts of carbohydrates, the creatin does not disappear, but is increased.

Wolf[9] has recently called attention to the fact that he has ob-

[8] A small amount of urine, though probably not more than 1/30 of the whole, was lost on this day, so that the correct figures would be even a little higher than those given.

[9] Wolf, *Jour. Biol. Chem.*, 1912, x, 473.

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served creatin in the urine in cases where carbohydrate was present in the diet, but the cases he refers to are those of persons suffering from typhoid fever and pneumonia.[10] In these diseases, there is, due to the infection and the high temperature, an increased destruction of body protein, and this destruction cannot be entirely prevented by the feeding of carbohydrates.

From the fact that there is, on the whole, rather more nitrogen excreted in the third period than in the second, the creatin of the meat in the food will suggest itself as one possible source of the creatin in the urine. But against the view that the creatin comes wholly from the meat are the following objections: (1) in the earlier observations creatin was found in the urine on a meat-free diet;[11] and (2) the amount does not vary with the amount of nitrogen excreted, but depends more on the amount of fat and carbohydrate in the diet, than on the amount of meat. This can be observed in the tables in several instances. It is seen, for example, in table III on the fifth, sixth, and tenth days. On the fifth day the patient had, among other things, 54 grams of chicken and there were 4.06 grams of nitrogen in the urine; on the sixth day the amount of chicken was raised to 70 grams and the nitrogen of the urine rose to 5.26 grams. Yet on both days the amount of creatin excreted was the same. On the ninth and tenth days, the nitrogen excretion was respectively a little below and a little above that of the sixth day; yet the creatin excretion for these days was higher, and on the tenth day it was more than twice as high. Still other examples may be found. These considerations exclude the possibility that either the whole or even a large part of the creatin of the urine depends on variations in the amount of the creatin in the food. But they do not exclude the possibility that the source of some of the urinary creatin is the creatin of the food.

The constancy in the excretion of the creatinin and the great variation in the excretion of the creatin in all these observations lend support to the conception that the metabolism of creatin and that of creatinin are independent of each other. Moreover, these observations are hardly in accord with the view that creatin and creatinin

[10] Ewing and Wolf, *Arch. Int. Med.*, 1909, iv, 330; Wolf and Lambert, *Arch. Int. Med.*, 1910, v, 406.

[11] McCrudden, *loc. cit.*

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are easily changed into each other in the organism, and that the sum of the two is of more importance than the amount of either one alone.

The results here presented are of importance with reference to their bearing on creatin and creatinin metabolism, but the physiology of these compounds is not at present well enough understood to enable us to show the bearing of the results on the nature of the processes going on in infantilism. Our findings are of considerable importance in relation to the cases under investigation, if we agree with Folin that creatin is a foodstuff and, when fed, may appear in the urine, provided the state of nutrition of the body is high, and the tissues are saturated with creatin. Rubner[12] has pointed out that two things are concerned in the process of growth: (*a*) the "tendency to grow," which is present only in immature animals and without which no animal can grow, however well nourished it may be; and (*b*) the foodstuffs which furnish the material for growth. Thus far in our investigations we have been unable to determine which of these two factors is at fault in the cases of infantilism of the type of Herter. If, however, Folin's view is confirmed, and the increased excretion of creatin on the carbohydrate-rich diet is taken to mean that the state of nutrition of the body is high and that for this reason a greater fraction of the creatin of the food passes through into the urine, the important conclusion must be drawn that the retarded development in these cases is due, not to an insufficiency in the materials for growth, but to the absence of the "tendency to grow."

Studies of other possibilities suggested by the results of the observations reported in this paper are now under way.

CONCLUSIONS.

In two cases of infantilism of the type of Herter, in one case of achondroplasia, and in one case of cretinism, creatin was constantly present in the urine and was increased in amount when the ingestion of carbohydrates was increased.

[12] Rubner, *Arch. f. Hyg.*, 1908, lxvi, 81.

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PURE CULTIVATION OF SPIROCHÆTA REFRINGENS.*

BY HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATE 69.

In 1905, in the article in which the discovery of *Treponema pallidum* was announced, Schaudinn and Hoffmann described a coarse spirochæta found in superficial lesions on the genitals. This organism was observed not only in syphilitic, but also in non-syphilitic lesions. It is differentiated from the pallidum by its larger dimensions, its plump shape, its comparatively few, irregular curves, and by its readiness to take various anilin dyes. The Giemsa solution stains it violet red. Schaudinn and Hoffmann named this organism *Spirochæta refringens,* and considered it non-pathogenic.

While the organism frequently comes under observation in examining scrapings for the diagnosis of syphilis, yet our exact knowledge of its biology has been fragmentary for the reason that no one had succeeded in isolating it in pure culture. Unquestionably, certain investigators have obtained a growth of this spirochæta in highly impure cultures, but a study based upon such contaminated material aided little in advancing our knowledge.

In my systematic attempt to secure pure cultures of various spirochætæ, I have succeeded in growing this organism in pure culture.

The strain of *Spirochæta refringens* that I cultivated was derived from the scrapings of a moist condyloma around the anus of a female patient. Under the dark-field microscope, the scrapings showed a moderate number of the refringens. The material was ground in a sterile mortar with sodium citrate solution, and the emulsion thus prepared was inoculated into several tubes of solid culture medium consisting of one part of ascitic fluid, two parts of slightly alkaline agar, and a piece of fresh rabbit kidney. The

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technique employed was the same as that for the cultivation of the pallidum[1] from a human being. After two weeks' incubation at 37° C. an impure culture was obtained. By the same process that was used for the pallidum and for the mouth spirochætæ, the refringens was finally purified.

The Properties of the Pure Culture (Figures 1 to 5).—Spirochæta refringens grows luxuriantly in the deeper part of a tube of ascitic tissue agar, and forms hazy colonies that gradually extend from the deeper parts of the tube to the upper. It is an anaerobic organism and no growth takes place within two centimeters of the surface of the medium. At 37° C., the growth becomes visible on about the fourth day after the inoculation, and proceeds for some weeks. It does not produce any visible changes in the medium, and the culture is free from any odor. Unlike the pallidum, a growth is obtainable in the medium without the addition of fresh tissue, although, like the microdentium, the growth is poorer when the tissue is absent.

In pure culture, *Spirochæta refringens* measures, on the average, about 0.5 to 0.75 of a micron in width, and about 6 to 24 microns in length. Chains of two, three, and four or more individuals may be seen. They are connected with one another by means of a thin filament. The number of curves varies with different individuals, but, as a rule, the middle part of the body is merely wavy, while the two extremities are more regularly and deeply curved. Not infrequently, however, the majority of the spirochætæ in certain cultures are fairly regularly curved throughout their entire length, the distance between two curves measuring about 2 microns. The ends of the organism are pointed, and to one or both of them is usually attached a fine, long, more or less stiff, but elastic projection, which has regular minute curves.

In a culture growing under optimum conditions, the spirochætæ are very motile. Under these conditions the curved flagella-like projections rotate with extraordinary rapidity around their point of attachment to the spirochætæ. While the flagella-like projection is rotating rapidly, the body of the spirochæta may remain motionless, or may be traversed by waves, convex curves becoming concave, and concave curves becoming convex. The waves begin at

[1] Noguchi, *Jour. Exper. Med.*, 1912, xv, 90.

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one end and may pass along the entire body, or may stop after having passed over only a few curves. Whenever motility is observed in the body of the spirochæta, the movements are repeated at frequent intervals. In a dead specimen, the curves are much more irregular and are often almost straightened out.

The body of the refringens was thought by Schaudinn to be ribbon-like, but in pure culture I find it to be cylindrical. In the refringens cultivated by me, there is no undulating membrane.

Regarding the mode of multiplication, I am still unable to decide whether or not a longitudinal division occurs before the spirochætæ are found attached end to end in pairs or in chains of several individuals. The ultimate division of these chain forms is transverse.

Pathogenicity.—Several attempts were made to produce lesions in the testicles of rabbits, or in the skin of *Macacus rhesus* monkeys, but thus far without a positive result.

GENERAL CONSIDERATION.

That *Spirochæta refringens* constitutes an independent species, and not a form representing merely a certain stage of *Treponema pallidum,* is proved by the present study and by my published observation on different strains of the pallidum in pure culture. Neither the refringens nor the pallidum lose their characteristics at any stage of cultivation. As a consequence, they may always be distinguished even in cultures.

A few investigators in questioning the evidence for the specificity of *Treponema pallidum* for syphilis have argued that, if the refringens is a form of the pallidum, the latter cannot be specific for syphilis, because the former is found in so many non-syphilitic lesions. As the two organisms are now shown to be different, this argument can no longer be employed.

Spirochæta refringens belongs, doubtless, to the genus *Treponema,* but, like the other members of this genus, it is entirely distinct from the pallidum.

There are still some important problems that await solution by means of pure cultures. Balanitis erosiva circinata is a clinically well defined disease, and in the lesion a large spirochæta resembling the refringens is usually found. Von Prowazek and Hoffmann

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considered this organism to be its causative agent. Mindful of the fact that the refringens has a wide distribution in the genital region, one naturally seeks a means by which the two morphologically allied forms, the refringens and balanitidis, can be definitely differentiated. This is possible only through the growth of both organisms in pure culture. Unfortunately, I have seen but few cases of this type of balanitis and for this reason I have been unable to attempt seriously its cultivation.

From the point of view of cultivation, another problem of interest is the relation between *Spirochæta buccalis* and the refringens. There is at present much controversy in regard to the identity of these spirochætæ. Some consider them identical, while others regard them as independent varieties. My numerous attempts to isolate the buccalis in pure culture have thus far been fruitless. There seems to be no doubt but that the buccalis is much more difficult to grow on artificial medium than the microdentium or macrodentium. In an impure culture of the buccalis, I was much impressed with the morphological similarity that it bears to the refringens, but the question of the identity of these two spirochætæ requires further study.

SUMMARY.

A strain of *Spirochæta refringens* has been obtained in pure culture, and its morphological and cultural characteristics have been studied. The strain possessed no pathogenicity for rabbits or monkeys. It probably belongs to the genus *Treponema*, but it is easily differentiated from *Treponema pallidum*, *Treponema microdentium*, and *Treponema macrodentium*. Its relation to *Spirochæta balanitidis* and *Spirochæta buccalis* is still undetermined.

EXPLANATION OF PLATE 69.

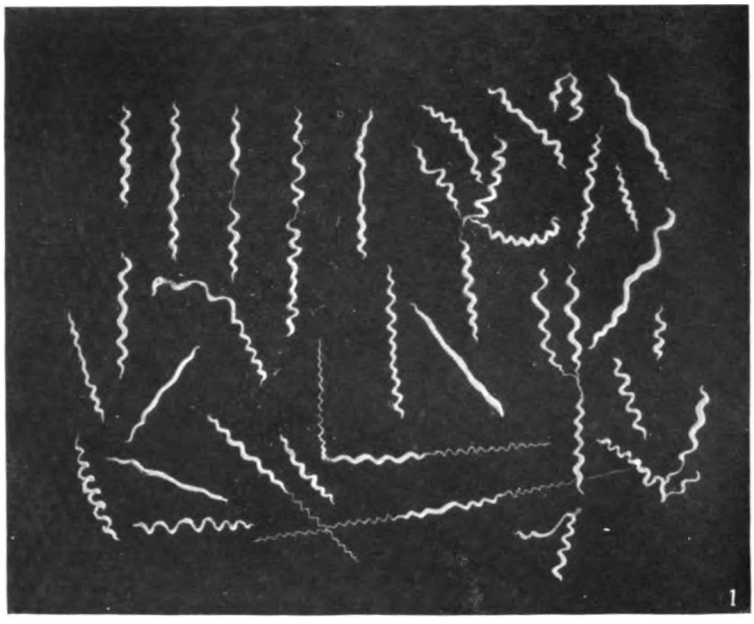
FIG. 1. A schematic drawing of *Spirochæta refringens* from pure cultures (dark-field).

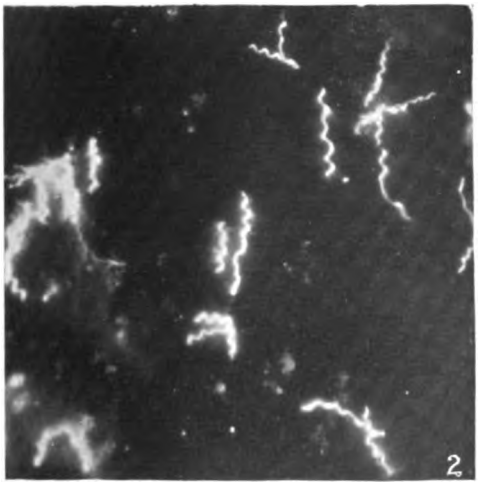
FIG. 2. *Spirochæta refringens* from a three weeks' old pure culture in ascitic tissue agar, at 37° C. (dark-field). × 1,100.

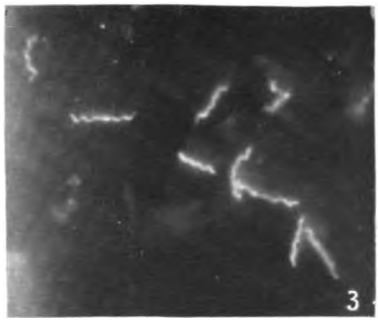
FIG. 3. *Spirochæta refringens* from a pure culture seven days old (dark-field). × 1,100.

FIGS. 4 and 5. *Spirochæta refringens* from a pure culture two weeks old (dark-field). × 1,100.

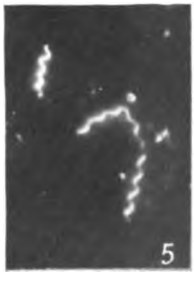
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ON THE PERMANENT LIFE OF TISSUES OUTSIDE OF THE ORGANISM

By ALEXIS CARREL, M.D.

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ON THE PERMANENT LIFE OF TISSUES OUTSIDE OF THE ORGANISM.*

BY ALEXIS CARREL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 75 AND 76.

INTRODUCTION.

It is known that the life of a tissue cultivated *in vitro* is very short.[1] Generally, in three to fifteen days after the preparation of the cultures their growth becomes less rapid and eventually stops completely. The tissues then die and the cells disintegrate.

The purpose of the experiments described in this article was to determine the conditions under which the active life of a tissue outside of the organism could be prolonged indefinitely. It might be supposed that senility and death of the cultures, instead of being necessary, resulted merely from preventable occurrences; such as accumulation of catabolic substances and exhaustion of the medium. The suppression of these causes should bring about the regeneration of old cultures and prevent their death. It is even conceivable that the length of the life of a tissue outside of the organism could exceed greatly its normal duration in the body, because elemental death might be postponed indefinitely by a proper artificial nutrition.

In the following experiments I sought not a complete solution of the problem of prolonging indefinitely the life of tissues *in vitro*, but the facts that would show in which direction we should proceed in order to secure a complete solution, if such a solution be possible.

In September, 1911, I tried to increase the duration of the life of cultures of connective tissue.[2] The cultures were washed for

* Received for publication, March 15, 1912.

[1] Harrison, Harvey Lectures, Philadelphia and London, 1911–1912; *Jour. Exper. Zoöl.*, 1910, ix, 787; Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

[2] Carrel, *Jour. Am. Med. Assn.*, 1911, lvii, 1611.

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several minutes in Ringer's solution and were then placed in a new medium. As a consequence, the growth was observed to become more rapid. By repeated washings and passages the occurrence of senility was prevented, and the length of life was very much increased. Some cultures could be maintained in active life for fifty, fifty-five, and even for sixty days.[8] These results showed that the early death of tissues cultivated *in vitro* was preventable, and, therefore, that their permanent life was not impossible.

Subsequently, I developed other techniques by which it might be possible to obtain permanent life of tissues. The ideal method would have been to give to the cultures an artificial circulation by which they might have secured their nutrition and eliminated their waste products. It was attempted to place the tissues in a thin layer consisting of cellulose and coagulated plasma, and through this a slow stream of serum flowed. But it appeared soon that better results could be obtained by a simpler and more indirect method. Instead of establishing a circulation of the medium through the tissues, it was easier to have the cultures pass from medium to medium. The media usually employed consisted of Ringer's solution and hypotonic plasma. It was found that the resistance of a tissue placed in a non-nutritive, but preservative medium was very much stronger if the temperature was low. The cultures were, therefore, placed under conditions having two alternate phases: there was a phase of active life at 38° C. during which the tissue grew, used its medium, and was surrounded by its catabolic products; and a phase of latent life at 0° C. during which it was washed free of its products, to be subsequently placed in a new medium.

In February, 1912, this technique was modified and the phase of latent life in Ringer's solution in cold storage was replaced by a phase of active life in serum in the incubator. Instead of being interrupted, the life of the tissues was continuous and their growth more rapid.

METHODS.

The methods chiefly employed were three. They are as follows: *Technique I.*—The original piece of tissue and the surrounding

[8] Pozzi, *Bull. de l'Acad. de méd.*, 1912, lxvii, 26.

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new cells were extirpated with a cataract knife from the fragment of coagulated plasma that contained them. They were washed for several minutes in Ringer's solution at the temperature of the laboratory, and were then placed in a new medium composed of one or two parts of distilled water and of three parts of normal plasma, to which a drop of embryonal or muscle extract was added. The washings and passage were made either before the appearance in the culture of the symptoms of senility, or when these were still very slight. The passage was repeated more or less frequently according to the rate of the growth and to the conditions of the cells.

Technique 2.—In the second method the washing of the cultures was more prolonged and was performed while the tissues were in cold storage. The life of the cultures was maintained under conditions in which two phases alternated: a phase of active life in a plasmatic medium in the incubator, and a phase of latent life in Ringer's solution in the refrigerator.

Technique 3.—In the third method the cultures were maintained in a condition of continuous life by alternating passages from plasma to serum at the temperature of the organism. The cultures were prepared in the following manner: A piece of silk veil about one centimeter square was placed on a cover glass and moistened with a drop of plasma. A fragment of tissue was deposited in the centre of the square and covered by a few drops of plasma. Afterwards the culture was sealed on a hollow slide, put in the incubator, and allowed to grow. After a few days the silk veil together with the coagulated plasma and tissue, was detached from the cover-glass with a knife. As the silk veil acted as a skeleton for the plasmatic jelly, the original fragment, and the new tissue cells, the culture could be handled easily without folding and retraction of the medium, and without deformation of the cells. The veil and the culture attached to it were placed in a tube containing homogenic serum, which was kept at incubator temperature. After a few days it was put again in plasma, and subsequently the two phases alternated regularly.

Experiments and Results.—A great many experiments were performed. In most of the cultures were used connective tissue of

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blood vessels, heart, skin, muscles, peritoneum, and spleen, of fourteen to twenty day old chick fetuses. In other cultures were employed fragments of the Rous sarcoma, and in a few cases, tissues of adult dogs. Many cultures survived six to fourteen or fifteen passages in a condition of interrupted or continuous growth. A few cultures were transplanted seventeen, eighteen, and even twenty or more times. Some are still living and have reached the beginning of the third month of their life *in vitro*. It was possible, therefore, to study the morphological and dynamic characters of tissues cultivated for more than two months outside of the organism.

MORPHOLOGICAL CHARACTERS.

The appearance of the cultures grown by technique 1 was almost identical with that of the cultures grown by technique 2. The fragment of tissue was surrounded rapidly by cells spreading in a thin layer through the medium. After section of the plasma, the new tissue retracted around the original fragment forming an opaque crown. During the period of washing there was no modification of the culture, but as soon as the culture was put in a new medium in the incubator, elongated cells began to grow from the edges of the old plasma and to spread into the new medium. After a few passages the original fragment appeared diminished in size and surrounded by a dense envelope of a greyish tissue from which radiated a great many elongated cells, living and dead.

The original fragment was often encircled by several concentric rings, each representing a period of active life. Soon the rings were united in a homogeneous tissue. When they remained distinct, however, they might indicate the number of passages undergone by the culture, as the age of a tree can be read on a section of its trunk. Progressively the original fragment disappeared and was replaced by an amorphous yellowish substance. The form of the culture was also modified. At the beginning of the growth the culture appeared like a thin plate, but it grew progressively more and more like a flattened sphere in the center of which were accumulated a few foreign bodies and pieces of old plasma (figure 1), which were encysted by the living cells. After about forty days the

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cultures of connective tissue generally took the typical appearance represented in the drawings (figures 2 and 3) of a culture fifty days old.

Cultivation of Connective Tissue (Experiment 715).—A short segment of the iliac vessels of a twenty-one day old chick fetus was extirpated on November 1, 1911, and placed in hypotonic chicken plasma and muscle extract. A large growth of connective tissue cells developed in the next few days.

On November 3 the plasma was cut and the culture was placed in Ringer's solution in cold storage from 9:52 A. M. to 10:35 A. M. Afterwards, the first passage into a new medium was made. A large growth resulted.

On November 6 the second passage was made after washing in Ringer's solution and keeping in cold storage from 9:55 A. M. to 10:58 A. M. At 2:30 P. M. fusiform cells had already invaded the new medium.

On November 9 the third passage was made after washing for six minutes in Ringer's solution, and on November 13 the fourth passage was made after washing in Ringer's solution and keeping in cold storage from 9:50 A. M. to 10:42 A. M. The iliac vessels were becoming progressively smaller, while a thick crown of cells in concentric lines surrounded the original fragment.

On November 16 the culture was washed in Ringer's solution and in cold storage from 9:20 A. M. to 10:12 A. M., and then placed in a medium composed of plasma and muscle extract. There was a large growth (fifth passage).

On November 20 the culture was washed in Ringer's solution in cold storage from 9 A. M. to 11:12 A. M., and then transferred to plasma and muscle extract (sixth passage).

On November 24 the culture was placed in a medium composed of hypotonic plasma and embryonic extract (seventh passage), after having been washed in cold storage and in Ringer's solution from 9 A. M. to 10:18 A. M.

On November 25 a large growth of cells was seen in the medium. The original fragment had almost completely disappeared, and the culture has a tendency to take a spherical form.

On November 28 the culture was placed in Ringer's solution and in cold storage from 9:30 A. M. to 12:02 P. M. and afterwards in hypotonic plasma and embryonal extract (eighth passage), and on November 29 a large growth was observed. The culture assumed the form of a flattened sphere, the center of which was clear and amorphous.

From December 2 to December 12 the culture underwent its ninth, tenth, eleventh, and twelfth passages. Before each passage it was washed for four or five minutes in Ringer's solution at the temperature of the laboratory.

On December 14 the culture was composed of a central amorphous part of a thick ring of new tissue, and of a peripheral crown of elongated cells radiating through the culture medium.

On December 15 the culture was placed into a new medium after having been washed for four minutes, and on December 16 a large growth through the new medium was seen. The rate of growth had markedly increased.

On December 18 the culture was washed for three minutes and underwent its fourteenth passage, in which a rapid growth of elongated tissue cells was observed.

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On December 20 the culture was fixed in formalin and stained with hematoxylin. Two drawings of the stained specimen were made (figures 2 and 3), showing the amorphous center and its crown of actively growing tissue.

Even after seventy-five or eighty days, cultures of connective tissue made by the first two methods had about the same appearance, all of them resembling the later stages of the culture of the iliac vessel first described.

By the same method a few fragments of adult dog tissues, such as thyroid and periosteum, were cultivated, and several generations of connective tissue cells were obtained.

Frequently the cultures of Rous sarcoma died after a few days. The plasma became liquefied and the tissue disintegrated. Nevertheless, a small fragment of it could be kept in active life for forty-six days.

Culture of the Rous Sarcoma.—On January 24, 1912, a small piece of a Rous sarcoma was cultivated in hypotonic plasma. The growth was rapid and abundant. On January 26 the fragment was surrounded by an immense number of radiating fusiform cells and by a peripheral crown of round and polygonal cells. On January 26 and 29 the culture was washed for three or four minutes in Ringer's solution and put in a new medium. On January 30 fusiform cells only could be seen in the culture. On January 30 and on February 1, 3, 5, and 7 the culture underwent its third, fourth, fifth, sixth and seventh passages. The growth was not very active. On February 7 the culture was divided into two parts. One of these was washed and underwent its eighth passage on February 9. After the ninth and tenth passages on February 10 and 12, a microbian colony appeared in the medium. Nevertheless the active part of the culture could be extirpated without being contaminated and underwent its eleventh passage on February 15.

Afterwards the growth was small but aseptic. After the twelfth passage on February 17 the tissue produced a partial liquefaction of the medium with division or dissociation of the new tissue. Nevertheless, after the thirteenth passage on February 20 and the fourteenth passage on February 22 the tissue began to grow more abundantly and regularly without liquefaction of the medium. From February 24 to March 1 the culture underwent its fifteenth, sixteenth, seventeenth, and eighteenth passages. The growth was abundant and the culture increased very much in size. On March 5 a marked retraction of the plasma occurred. After the nineteenth and twentieth passages the growth became very small and death occurred without any apparent cause.

When the tissues were cultivated according to the third method the appearance of the cultures was different. Instead of being interrupted, the growth was continuous, and as there was no sectioning of the plasma, there was, as a consequence, no retraction.

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Moreover, as the plasma was supported by its skeleton of silk, the culture could not take a spherical form, and the tissue grew in a thin layer; that is, under the best conditions for a tissue deprived of circulation. The plasmatic jelly was adherent to the silk veil, and the tissue imbedded in the plasma grew as in an ordinary culture. The cells could use the silk threads as a support, but they seldom did. The purpose of the silk was merely to give such a consistency to the medium that it would not fold over or become spherical during the passages in plasma and in serum.

Normal heart was cultivated according to this technique. Its appearance after a few passages was the same as after a few days. The size of the growth alone was modified.

By the same procedure many fragments of the Rous sarcoma were also cultivated. They could be kept in a condition of active and continuous development for more than fifteen days. But the medium was progressively overcrowded with dead cells and the growth of the tissue became more irregular and less abundant. The fragments could not adapt themselves to their new conditions of life and were less resistant than normal connective tissue.

The appearance of the cells growing in cultures of normal connective tissue often varied periodically. During the first few days of growth the fusiform cells were long and thin and contained clear cytoplasm. After four or five days they became larger and their cytoplasm was darker and filled with large fatty granulations. At the same time the rate of growth diminished. After being washed and transferred to a new medium, the appearance of the cells was again modified. They regained their original appearance and the rate of growth was accelerated. Instead of being periodical, the morphological modifications might be continuous. For instance, in the cultures of spleen the original fragment was surrounded during the first phases of active life by an envelope of ameboid cells. After a few days long chains of fusiform cells appeared around the fragment, while the ameboid cells died and disappeared. Ultimately the fusiform cells were replaced by small polygonal cells which spread around the fragment in a continuous layer. In the culture of the Rous sarcoma the round cells disap-

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peared after a few days, while the elongated cells were still growing after forty days. In a culture of heart very large ameboid cells wandered out from the central fragment for more than two months. At the beginning of the third month of its life the culture was composed of elongated cells radiating through the old plasma. They were apparently the result of the development of the large ameboid cells.

DYNAMIC CHARACTERS.

The rate of growth could be appreciated by the rapidity with which the cells, after a passage, appeared and spread in the new medium. The nature of the medium, its osmotic tension, the way in which the plasma was cut, the amount of old plasma left around the cells, the form of the culture, and the frequence of the passages, often had a marked influence on the rate of the growth. The oscillations in the rate seemed generally to be the result of accidental causes, but it was very difficult to exclude the possibility that they might be due to causes inherent in the condition of tissues liberated from the control of its organism. Generally, after the first passage the cells appeared in the medium without a latent period. This was true even in the culture of periosteum and of other tissues of adult dogs. Two or three hours after a passage many elongated cells were observed growing out from the edges of the old plasma into the new medium, and they soon surrounded the central piece by a dense layer. The rapidity of their growth often diminished after a few passages, but afterwards increased progressively. The rate of growth of a culture forty or forty-five days old was more rapid than when it was ten or fifteen days old. It seemed that the older a culture was, the quicker it grew. The rate of growth of a fifty-four day old culture of connective tissue from the portal vein was more rapid than at the beginning of its life *in vitro*. The rate of growth of a culture of heart was greater at the beginning of the third month of its life outside of the organism than at any other period.

These accelerations of the growth were possibly functions of the age of the culture. But the number of the experiments was not large enough to exclude the possibility that they were due to other causes.

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The size of the cultures underwent also many modifications. For long periods of time, the cultures increased in size, diminished, or were not modified.

The diminutions in size were due chiefly to accidental causes, such as destruction of cells during the handling of the cultures, folding of the plasma, and concentration of the medium, but especially to microbian infection.

In a few experiments marked increase in size was observed. The tissue of a culture of portal vein had been considerably réduced after infection, and for several generations it increased but slightly. Then the growth became more active and after the fiftieth day the volume of the tissue increased rapidly. In a culture of connective tissue which had remained very small for two months, a great acceleration of growth and a marked increase in size was observed after sixty days. In another instance a culture of heart became so large after sixty-two days that it had to be divided into several parts.

It is generally difficult to ascertain whether or not the mass of the tissue actually increases, because changes in the density of the tissue can be mistaken for a change in its mass. Nevertheless, the observation of a culture of a diluted suspension of cells demonstrated that a real increase in the volume of the tissue could take place.

Culture of Cells Suspended in Ringer's Solution.—On February 1, 1912, a nine day old chick embryo was cut into very small pieces, and to the pieces an equal volume of Ringer's solution was added. The suspension was centrifugalized for five minutes, and a drop of the supernatant fluid was then added to a culture medium. On February 2 many isolated cells were seen in the peripheral part of the medium. On February 5 the number of these cells had increased very much. The part of the plasma where they had grown was resected, washed, and put in a new medium. The culture underwent its second, third, fourth, and fifth passages on February 2, 10, 13, and 16. The cells had then increased so much in number that they formed a real tissue, and had the appearance of an ordinary culture. The life of these cells afterwards was like that of other cultures.

Since a few scattered cells could generate a culture similar to the culture of fragments of tissue, it is absolutely certain that the cells multiply and that they do not merely wander from the central piece.

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Generally, the cultures remained very small in spite of their constant growth, and often they did not increase at all in size. It is probable that the limitation of growth is due to certain mechanical conditions. Cultures have a tendency to become spherical. The nutriment that reaches the center of such cultures is insufficient, but that which is supplied to the periphery is abundant. Hence, the cells at the periphery multiply ceaselessly. But as soon as cells are isolated from the medium by a sufficient thickness of new tissue, their nutrition probably becomes deficient and they disintegrate. While the culture increases constantly by its periphery, it diminishes in its center and therefore its volume does not increase.

A tissue deprived of circulation probably cannot grow in a nutritive medium beyond a certain volume which is a function of its permeability to the medium and consequently of the thickness of the culture. If the culture be spherical its volume will remain very small, since the nutrition of its center is insufficient. From a theoretical standpoint, on the other hand, a tissue growing in a very thin layer could increase indefinitely. It is for this reason that the tissues were cultivated on a silk veil, for the veil prevented the plasma of the culture from retracting and becoming spherical.

The attempt was also made to ascertain whether tissues after a long period of life *in vitro* could retain their normal functions. The experiments were carried out with fragments of chick fetus heart, which, as is well known, can grow and pulsate for several days when kept at a proper temperature in plasma. In December, 1911, it was observed that when the pulsations of a culture of heart stopped, they began again after the tissue had been washed and transplanted into a new medium. Then in January, 1912, the attempt was made to determine the interval during which the cardiac muscle would retain its property of contracting rhythmically. Three experiments gave positive and identical results. One of these will be described.

Cultivation of the Heart (*Experiment 720-1*).—On January 17, 1912, a small fragment of the heart of an eighteen day old chick fetus was cultivated in hypotonic plasma. The fragment pulsated regularly for a few days and grew extensively. After the first washing and passage on January 24 the culture grew again very extensively, but there were no rhythmical contractions. On

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January 29 and February 1, 3, 6, 9, 12, 15, 17, 20, 24, and 28, the culture underwent eleven washings and passages. It became surrounded by fusiform cells and many dead cells. There were no pulsations. After the twelfth passage the culture did not grow at all. Then the tissue was dissected and the old plasma was completely extirpated. A small central fragment was removed, washed, and put in a new medium. On March 1 it was pulsating at a rate that varied between 60 and 84 per minute. On March 2 the pulsations were 104 at 41° C., and on March 3, 80 at 40° C., but on March 4 the pulsations were very weak and stopped altogether at 2 P. M. On March 5 the culture underwent its fourteenth passage, and the pulsations reappeared immediately. They became weak again on March 2. On March 8 the fifteenth passage was made. On March 9 the pulsations were again 80 to 82 per minute at 40° C., and on March 12 they were 60 per minute. They then became slower and weaker. After the sixteenth passage on March 12 the pulsations were irregular, and the fragment beat for a series of 3 to 4 pulsations, and then stopped for about 20 seconds. After the seventeenth passage on March 16 regular pulsations at 52 beats per minute reappeared, and the tissue grew abundantly. After the eighteenth passage on March 19 the pulsations were irregular.

It was thus demonstrated that a fragment of chicken heart could still pulsate rhythmically at the beginning of the third month of its life outside of the organism. It showed, consequently, that a fragment of tissue living *in vitro* could retain its normal function for a long time.

The maximum age that tissues living *in vitro* can reach is still undetermined. Many cultures have died after less than two months, but a few were very active at the beginning of the third month of their life outside of the organism. Generally, the tissues seemed, after a time, to adapt themselves to their new condition, and after the fourteenth or fifteenth passage, very few cultures died spontaneously. In the handling and changes required by the passages, the cultures were exposed to many accidents, chiefly to microbian infections. In case of a local infection the part of the culture that was not yet infected was resected with a cataract knife and placed in a new medium. Often the culture recovered and produced several generations of cells that were free from microbian infection. But when infection was generalized, the tissues always died rapidly. Many cultures died of sepsis. In the description that follows is an illustration of this kind of death.

Culture of Portal Vein (Experiment 483).—On September 27, 1911, a fragment of the portal vein of a chick fetus sixteen days old was cultivated in three

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parts of chicken plasma and two parts of distilled water. The tissue grew very extensively. On October 2 the culture was divided into three parts. One was washed in Ringer's solution for five minutes and put in a new medium. On October 7 it had grown extensively and was again divided into two parts. One part was washed and transplanted into a new medium and a large growth followed. On October 9 and 12 the third and fourth washings and passages took place. On October 14 a very large growth of long, clear, slender fusiform cells was present. On October 16 the growth had stopped and the cells were large and contained very large fat granules. On October 16 the fifth washing and passage took place, and on October 17 the growth of fusiform cells, which were again clear with few granules, was very active. After the sixth passage on October 20 retraction of the medium occurred, and the growth of the tissue was slight. By October 22 the original fragment had diminished greatly in size. On October 23 and October 26 the culture underwent its seventh and eighth passages, and on October 27 the original fragment was no longer to be distinguished. The culture was composed of an amorphous central part around which a crown of active tissue had developed. The tissue was disposed in concentric circles and from its peripheral part elongated cells radiated through the culture medium (figure 1). After the ninth and tenth passages on October 30 and November 4, a great increase in the rate of the growth was observed, but after the eleventh passage on November 9 the culture assumed a spherical shape, but grew extensively nevertheless. On November 10, 13, 16, and 18, the twelfth, thirteenth, fourteenth, and fifteenth passages took place. The rate of growth was very rapid. One hour after the fifteenth passage, the fusiform cells had already invaded the new culture medium, but on November 19 a large microbian colony appeared on one side of the culture, and on November 20 the culture was invaded by a great many bacterial colonies. The death of the tissue occurred soon after.

Nearly all the cultures made in the latter part of 1911 died in the same manner after one to two months. By slight modifications of the technique, however, infection was almost completely eliminated in the experiments made in the beginning of 1912.

Of sixteen cultures of heart and blood-vessels made on January 17, 1912, five were still very active in March, 1912, and of the five active ones, two heart cultures previously described grew slowly, but pulsated, and another heart culture, which pulsated from time to time, produced a large growth of ameboid and fixed cells which covered an extensive area of the medium. In this instance after having been motionless for two months, the central part of the culture manifested strong rhythmical contractions on the sixty-fifth day of its life *in vitro*. Moreover, two cultures of connective tissue made on January 17 were growing actively at the beginning of April. The rate of growth and the increase in their volume became very much greater as they grew older.

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SUMMARY AND CONCLUSION.

In two series of experiments made at the end of 1911 and at the beginning of 1912, new techniques were developed with the view of investigating the problem of prolonging indefinitely the life of tissues isolated from the organism. These techniques are far from perfect and will doubtless be modified in the future. But they have already permitted the establishment of new facts.

Fragments of connective tissue have been kept *in vitro* in a condition of active life for more than two months. As a few cultures are now eighty-five days old and are growing very actively, it is probable that, if no accident occurs, the life of these cultures will continue for a long time.

In some cases the rate of growth of the tissues increased in direct ratio to the age of the culture.

Fragments of heart pulsated rhythmically at the beginning of the third month of their life *in vitro*.

These facts show that experiments made with these or with more perfect techniques and followed over long periods of time may lead to the solution of the problem of permanent life of tissues *in vitro*, and give important information on the characters acquired by tissues liberated from the control of the organism from which they were derived.

EXPLANATION OF PLATES.

PLATE 75.

FIG. 1. A thirty day old culture of connective tissue. The center of the culture consists of debris of old plasma. Around it is a ring of concentric layers of very active new tissue.

PLATE 76.

FIG. 2. A fifty day old culture of connective tissue. Active tissue is encircling a piece of old plasma.

FIG. 3. Peripheral part of the same culture.

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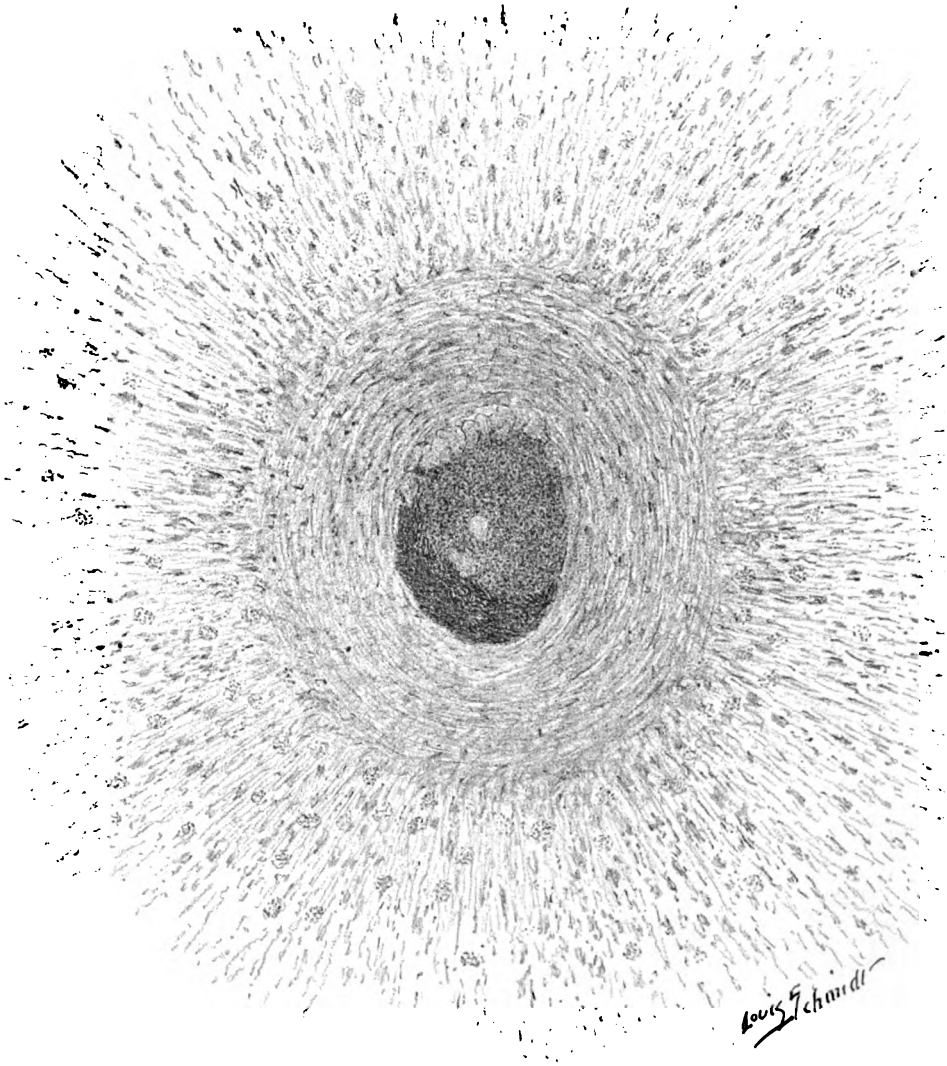


FIG. 1.

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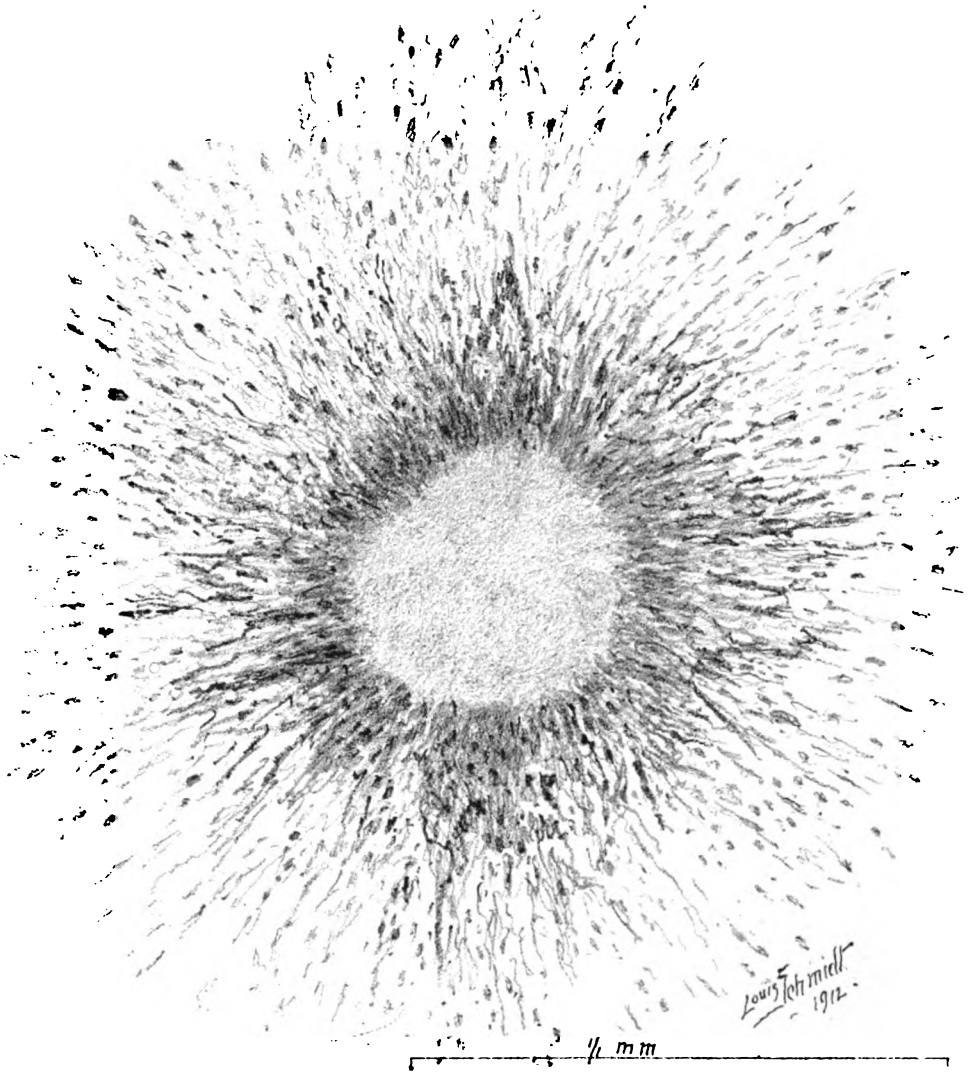


FIG. 2.



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Benzol als Leukotoxin.

Studien über die Degeneration und Regeneration des Blutes und der hämatopoetischen Organe.[1])

Von

Dr. med. Laurence Selling.

Aus dem pathologischen Laboratorium der Johns Hopkins-Universität.

Hierzu Tafel I—III und 6 Figuren im Text.

Die Untersuchung, über welche im folgenden berichtet werden soll, wurde veranlaßt durch eine Reihe von Fällen, die im Jahre 1909 unter Dr. L. F. Barker im Johns Hopkins-Hospital zur Beobachtung kamen. Vom klinischen und pathologischen Standpunkt aus ist über die Fälle bereits im „Bulletin of the Johns Hopkins-Hospital" (48) berichtet worden; doch seien die Punkte, die hauptsächlich von Interesse sind, hier kurz wiedergegeben.

Die Patienten, um die es sich handelt, waren drei vierzehnjährige Mädchen, die in ein und demselben Raum einer Zinnbüchsenfabrik beschäftigt waren. In dem betreffenden Raum wurde eine besondere Art von Büchsen hergestellt, bei welchen als Ersatzmittel für Lot, Kautschuk verwandt wurde und zwar wurde reiner Kautschuk zugleich mit einem Mineralfarbstoff und einem Harz in käuflichem Benzol aufgelöst. Wie die in der Folge ausgeführten Versuche ergaben, war das Benzol für die Vergiftungserscheinungen, welche auftraten, verantwortlich; dabei machte es keinen Unterschied, ob das unreine Benzol des Handels oder ob das chemisch reine Produkt (C_6H_6) verwandt wurde. Die Aufnahme des toxischen Materials erfolgte im wesentlichen durch die Lunge.

Zwei von den drei dem Hospital überantworteten Fällen endeten tödlich. Die beiden Patientinnen waren 4—5 Monate lang in der Fabrik tätig

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[1]) Ausgeführt mit einer Unterstützung aus dem Fonds des „Rockefeller Institute for Medical Research".

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gewesen und hatten die ersten Symptome etwa einen Monat vor ihrer Aufnahme bemerkt.

Die wesentlichsten klinischen Erscheinungen waren:

I. Purpura haemorrhagica mit typischen Hauterscheinungen, Blutungen aus den Schleimhäuten und Netzhauthämorrhagien.

II. Ein Blutbild, das in der Hauptsache mit dem einer aplastischen Anämie übereinstimmte:

a) Leukopenie, die am meisten auffallende Erscheinung bei der Blutuntersuchung. In dem einen Fall betrug die Zahl der Leukocyten bei der Aufnahme 1280 pro cmm und sank vor Eintritt des Todes auf 480 bis 600 pro cmm; im zweiten Fall wurden bei der Aufnahme 560, vor dem Tode 140 Leukocyten im cmm Blut gezählt.

b) Relative Verminderung der polymorphonukleären Elemente (43 bzw. 18 %) und relative Vermehrung der mononukleären Elemente (55 bzw. 81 %).

c) Anämie. Im ersten Fall waren 640000, im zweiten 1150000 rote Blutkörperchen im cmm Blut enthalten; die entsprechenden Hämoglobinwerte (Sahli) waren 8 und 15 %.

d) Nur geringe Veränderungen im Aussehen der roten Blutzellen. Geringe Anisocytose und Blässe; keine Poikilocytose.

e) Spärliches Auftreten von Blutplättchen.

f) Abwesenheit regenerativer Formen. Es wurden keine Myelocyten und nur eine kernhaltige rote Zelle (ein Megaloblast) gefunden.

Die Patientinnen befanden sich bei ihrer Aufnahme scheinbar in ziemlich gutem Zustande, doch verschlimmerte sich ihr Befinden stetig, wobei Symptome schwerer Toxämie (hohes Fieber, rascher Puls, Benommenheit) auftraten, während gleichzeitig sowohl die Purpura wie auch die Anämie und Leukopenie ständig zunahmen. Der Tod trat 6 bzw. 7 Tage nach der Aufnahme ein.

Benzol (Benzen), C_6H_6, ist ein Produkt der fraktionierten Destillation des Steinkohlenteers; doch ist das im Handel befindliche Produkt gewöhnlich ein unreines Material, welches außer C_6H_6 noch in wechselnden Mengen Toluol und Xylol, sowie geringe Mengen von Schwefelkohlenstoff, Ihiophen usw. enthält. Es ist zu unterscheiden von Benzin, einem Produkt, nicht des Steinkohlenteers, sondern aus Rohpetroleum, welches in der Hauptsache aus Hexan und Heptan besteht.

Das Studium klinischen Materials hat ergeben, daß Benzol ein stark leukotoxisches Agens ist; es zerstört die Leukocyten des zirkulierenden Bluts und verursacht eine schwere Schädigung des Knochenmarks. Die experimentelle Untersuchung zeigt aber, daß die Wirkung des Giftes sich nicht auf das zirkulierende Blut und das Knochenmark beschränkt, sondern daß das gesamte hämatopoetische System — Knochenmark, Lymphdrüsen, Milz und lymphadenoides Gewebe des Darms — betroffen wird. Alle diese Organe werden geschädigt, und die Veränderungen, welche in ihnen auftreten, sind von ähnlicher Art, wenngleich, wie sich zeigen wird, Unterschiede quantitativer Natur bestehen. Das myeloide Gewebe wird in höherem Maße geschädigt als das lymphadenoide Gewebe, und in Übereinstimmung hiermit sind die polynukleären Elemente des Blutes stärker betroffen als die nichtgranulären mono-

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nukleären Elemente. Die kernhaltigen roten Zellen des Marks werden zerstört, aber die zirkulierenden Erythrocyten werden in verhältnismäßig geringem Maße angegriffen.

Die vorliegende Arbeit berichtet über die experimentelle Untersuchung der Vergiftung durch Benzol unter besonderer Berücksichtigung: 1. des Verlaufs und der Ausdehnung der degenerativen Veränderungen, welche in den weißen Zellen des zirkulierenden Blutes und den Parenchymzellen der hämatopoetischen Organe auftreten. 2. Der Frage, wie schwer die hämatopoetischen Organe geschädigt werden können, ohne die Fähigkeit, sich vollständig wieder zu erholen, einzubüßen. 3. Des Verlaufs der regenerativen Veränderungen.

Das Studium der regenerativen Veränderungen in der Milz hat interessante Tatsachen gefördert, die betreffen: a) myeloide Metaplasie, b) die Umwandlung von Erythroblasten in Erythrocyten durch Kernausstoßung.

I. Literatur.

A. Blut.

Wenngleich eine detaillierte Besprechung der ausgedehnten modernen Literatur über Blut hier nicht erfolgen kann, so seien doch die wesentlichsten Tatsachen und Theorien, die für die Untersuchung in Betracht kommen, kurz zusammengestellt.

a) Entwicklung von Blutzellen.

Über den Ursprung der Blutzellen bestehen hauptsächlich zwei Theorien, die monophyletische und die polyphyletische. Die Verfechter der monophyletischen Theorie nehmen an, daß alle Zellen des Blutes und der hämatopoetischen Organe von einer gemeinsamen Mutterzelle stammen, entweder einem großen Lymphocyten (MAXIMOW (30, 31), SAXER (45), DOMINICI (13), PAPPENHEIM 42), oder von einer noch weniger differenzierten, etwas hypothetischen Lymphoidzelle (WOLFF (56)). Die Vertreter der polyphyletischen Theorie erklären dagegen, daß für die verschiedenen Zelltypen verschiedene Mutterzellen existieren, doch stimmen die einzelnen Autoren weder in ihrer Ansicht über die Zahl dieser Mutterzellen, noch in ihrer Meinung über die von jeder einzelnen Mutterzelle sich ableitenden Zellgruppen miteinander überein. Die Mehrzahl glaubt, daß zwei Mutterzellen existieren (EHRLICH (14), NÄGELI (38), SCHRIDDE (46)), und daß von der einen die Zellen der Myeloidreihe — Granulocyten, Erythroblasten und Megacaryocyten — und von der anderen die Zellen der lymphatischen Reihe sich ableiten.

Unter Anwendung einer Modifikation der ALTMANN'schen Methode fand SCHRIDDE (47), daß der große Lymphocyt — das Hauptelement der Keimzentra der Lymphdrüsen — sich von dem Myeloblasten — der Ursprungszelle der Granulocyten — durch eine spezifische Granulation in seinem Protoplasma scharf differenzieren ließ; er lieferte auf diese Weise neues Material für die Annahme der polyphyletischen Theorie. NÄGELI (38), tritt dieser Auffassung bei und glaubt, daß die Granulocyten und Lymphocyten verschiedenen Ursprungs sind; er basiert diese Anschauung nicht nur auf den ge-

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nannten Unterschied in der Granulation der Mutterzelle, sondern auch auf Verschiedenheiten in biologischen Reaktionen. Im übrigen sind aber die Befunde SCHRIDDE's recht skeptisch aufgenommen worden (BUTTERFIELD, MEYER, HEINECKE (6), MAXIMOW (33), WEIDENREICH usw.).

Neuere Untersuchungen, die MAXIMOW (30, 31, 33) am Kaninchen und DANTSCHAKOW (9, 10) am Huhn ausführte, haben die monophyletische Theorie wesentlich gestützt. Die beiden genannten Beobachter haben die embryonale Entwicklung von den frühesten Stadien an verfolgt und finden, daß die gemeinsame Mutterzelle des Blutes, ebenso wie das Endothel der primitiven Blutgefäße, von den Elementen des Mesenchyms abstammt. Diese Mutterzelle ist identisch mit dem großen Lymphocyten des späteren Lebens. Zuerst differenziert sie sich fast ausschließlich in der Richtung der primitiven erythroblastischen Elemente. Aus den großen Lymphocyten und direkt aus den Mesenchymzellen bilden sich überall in den Geweben primäre Wanderzellen. Nachdem die Bildung von primitiven Erythroblasten ziemlich weit vorgeschritten ist, beginnen sich diese Wanderzellen zunächst zu definitiven Erythroblasten und später zu Granulocyten zu differenzieren. Die definitiven Erythroblasten entwickeln sich im ganzen Bereich des Mesenchyms in Form umschriebener Inseln. Später erscheinen Granulocyten; sie treten zuerst isoliert auf, und liegen meist abseits von den Erythroblasteninseln. Die Lymphocyten entstehen, ebenso wie diese anderen Zelltypen, aus den primären Wanderzellen.

Die Arbeit bestätigt in mancher Hinsicht die Ansicht SAXER's (45), daß die primitiven Wanderzellen alle Zellen des Bluts und der hämatopoetischen Organe entstehen lassen. Die kernhaltigen roten Zellen entstehen in Form von deutlichen Zellinseln im Bereich des Bindegewebes; die Granulocyten bilden sich entweder einzeln oder in Zellgruppen. Die granulocytischen und erythroblastischen Herde liegen voneinander getrennt.

Die Entwicklung von Blutzellen in Form von Gruppen oder Inseln ist nicht nur im embryonalen, sondern auch im postembryonalen Leben beobachtet worden. MAXIMOW (30) hat beim Studium der Entwicklung des Knochens und Knochenmarks in der Niere (Kaninchen) nach Unterbindung der großen am Hilus eintretenden Blutgefäße gefunden, daß, während das myeloide Gewebe sich in der Markhöhle entwickelt, die Granulocyten und Erythroblasten nicht in einer wirren Masse, sondern als voneinander getrennte Gruppen und Inseln auftreten. WEIDENREICH (55) sagt (p. 395): „Während in einzelnen Blutnestern allerdings rote und weiße Blutkörperchen in allen Entwicklungsphasen nebeneinander getroffen werden, bieten doch sehr viele ein einheitlicheres Bild, so daß sich Gruppen der roten und weißen Entwicklungsreihen häufig voneinander getrennt festhalten lassen."

BUNTING (4, 5) kommt auf Grund von Versuchen mit Ricin und Saponin, zwei Substanzen, welche das Knochenmark schwer schädigen, zu der Auffassung, daß „das rote Mark des Kaninchens normalerweise im wesentlichen aus einem Mosaik von Zellen besteht, die in Gruppen oder sprossenden Zentren angeordnet sind; wobei einzelne Gruppen sich aus indifferenten Myeloblasten ... umgeben von Myelocyten und ... reifen polynukleären Zellen zusammensetzen, andere aus unreifen roten Zellen oder sogar aus indifferenten Myeloblasten ... mit umgebenden Schichten von Megaloblasten, und Normoblasten, und mit peripheren Schichten reifer roter Zellen.

b) Regeneration des Knochenmarks.

In der späteren Zeit der embryonalen Entwicklung enthält das Knochenmark sehr große Mengen von Elementen der jüngsten Typen, 60—70 (20)

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Myeloblasten (NÄGELI) und viele Megaloblasten. In dem Maße, wie die Entwicklung fortschreitet, verringert sich die relative Zahl dieser jüngeren Elemente, und im Leben des Erwachsenen sind normalerweise Myelocyten, polymorphonukleäre Leukocyten und Normoblasten die im Mark vorherrschenden Zellen. Der Zellgehalt des Marks wird aufrechterhalten durch die ständige Proliferation der differenzierteren Zellen. Tritt ein außergewöhnlicher Bedarf nach gesteigerter Zellerzeugung ein, so sind sie es, welche ihn befriedigen (TÜRK (53)). Das hyperplastische Mark der Pneumonie und das, welches nach schwerer Blutung auftritt, sind Beispiele dieses Typus von Proliferation.

Bei perniciöser Anämie liegen die Verhältnisse anders. Es erfolgt eine Umkehr zum embryonalen Typus. Sie besteht in einer erneuten starken Zunahme der Zahl der Myeloblasten und Megaloblasten, sei es infolge einer zu schweren Schädigung des Marks, sei es, daß die Anforderungen, die an dasselbe gestellt werden, zu lange Zeit hindurch sich geltend machen, oder sei es, daß diese beide Faktoren gleichzeitig wirken. Bei einigen solcher Fälle von perniciöser Anämie kann sich das Mark aus nicht ersichtlichen Gründen erschöpfen und gegenüber den an seine Leistungsfähigkeit gestellten Anforderungen vollständig versagen; es sind dies die Fälle von aplastischer Anämie. HIRSCHFELD (58) hat über ähnliches in einem Falle von Purpura haemorrhagica berichtet. BLUMENTHAL und MORAWITZ (3) ist es gelungen, analoge Verhältnisse bei Tieren herbeizuführen durch Erzeugung wiederholter Blutungen während einer langen Zeitperiode.

Die Bedingungen für die Erschöpfung des Marks sind verhältnismäßig selten vorhanden, denn normalerweise besitzt das Knochenmark bemerkenswerte regenerative Fähigkeiten. Beim Menschen vermag es, wie wir wissen, einen großen Verlust an Blutzellen auszugleichen. Auf experimentellem Wege hat HEINEKE gezeigt, daß das Mark, wenn es durch Röntgenstrahlen fast völlig aplastisch gemacht worden ist, sich innerhalb von 3 bis 4 Wochen wieder gänzlich erholen kann. Dabei sind es die jüngeren Elemente des Knochenmarks, die Myeloblasten, welche zuerst wieder auftreten.

Im allgemeinen ist die ausgleichende Funktion des Marks keine spezifische; es reagiert weder auf den Verlust an weißen Blutzellen ausschließlich durch Erzeugung von Granulocyten, noch auf den Verlust an roten Blutzellen ausschließlich durch Erzeugung von Erythroblasten. Wie SCHRIDDE (46) darlegt, scheint die Regeneration der Blutzellen unabhängig von dem Bedürfnis zu erfolgen, und es ist nicht leicht, im einzelnen Falle vorherzusagen, in welchen Mengenverhältnissen Granulocyten und Erythroblasten sich finden werden. BLUMENTHAL und MORAWITZ (3) erklären dies durch die Annahme einer Wechselbeziehung zwischen den beiden Gewebstypen.

c) Umwandlung von Erythroblasten und Erythrocyten.

Nach KÖLLIKER (25) und NEUMANN (40, 41), die als die ersten dies Problem diskutierten, erfolgt die Umwandlung des Erythroblasten in den Erythrocyten durch intracelluläre Auflösung des Kerns. Zweifel an dieser Auffassung traten auf, als RINDFLEISCH (43) beobachtete, daß in frischem Blut, zu welchem 0,6 % Chlornatriumlösung hinzugefügt war, der Kern aus dem Zellkörper ausgestoßen wurde. Die Forscher, welche seit RINDFLEISCH auf diesem Gebiete arbeiten, haben entweder eine dieser beiden Theorien angenommen, oder aber sie haben sich auf einen Mittelstandpunkt gestellt und erklären, daß beide Prozesse physiologischerweise vorkommen (BLOCK (2)).

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Für diejenigen, welche die Theorie der intracellulären Degeneration des Kerns vertreten, ist der Vorgang entweder eine allmähliche Auflösung des ganzen Kerns (Karyolyse), oder eine Zerstückelung des Kerns (Karyorrhexis) mit darauffolgender Auflösung der Fragmente. ISRAEL und PAPPENHEIM (24), welche diese Theorie vertreten, nehmen an, daß die Bilder, welche die Ausstoßung des Kerns zu zeigen scheinen, Kunstprodukte sind und hervorgerufen werden durch die Ausübung von Druck bei der Herstellung von Deckglaspräparaten oder, wie bei RINDFLEISCH's Beobachtung, durch den Zusatz 0,6 % NaCl-Lösung. Sie geben die Anwesenheit einer geringen Anzahl nackter Kerne zu, erklären dieselbe aber durch Annahme einer Degeneration des Protoplasmas, welche den Kern zurückläßt.

Die Autoren, welche die Ausstoßungstheorie annehmen, verweisen auf die Gegenwart nackter Kerne im Blut und den hämatopoetischen Organen, und erkennen die Erklärung von ISRAEL und PAPPENHEIM nicht an. Sie verweisen auf die sowohl in sorgfältig hergestellten Blutabstrichpräparaten wie auch in Schnitten fixierten Gewebes anzutreffenden Bilder, welche die verschiedenen Stadien der Kernausstoßung zeigen: eine mehr und mehr exzentrische Lage des Kerns; partielle Ausstoßung, wobei ein Teil des Kerns innerhalb, ein Teil außerhalb der Zelle liegt; vollständige Ausstoßung, bei welcher der Kern sich nahe der Zelle findet, aus der er stammt. Zwecks Widerlegung der Einwände von ISRAEL und PAPPENHEIM, die von RINDFLEISCH beobachteten Erscheinungen seien künstlich durch die Gegenwart von NaCl-Lösung herorgerufen, untersuchte WEIDENREICH (55) Knochenmarkzellen in Blutserum; dabei vermochte er RINDFLEISCH's Beobachtungen zu bestätigen.

Die Bilder von Karyolyse und Karyorrhexis, welche die Vertreter der intracellulären Theorie in ihrem Sinne deuten, werden von einigen der strikten Anhänger der Ausstoßungstheorie für Resultate ungenügender Fixierung gehalten; doch geben die meisten zu, daß es sich um physiologische Prozesse handelt, die der Ausstoßung vorangehen. Die Kerne, welche ausgestoßen werden, sind nicht junge, gesunde Exemplare, sondern solche, die schon Veränderungen — Pyknose, Schrumpfung, Karyorrhexis oder partielle Karyolyse — erlitten haben. Nach Zerstücklung des Kerns können die Einzelfragmente für sich ausgestoßen werden (WEIDENREICH (55)), und kleine Teilchen können sogar eine Zeitlang im Zellkörper verbleiben.

ALBRECHT, dessen Auffassung auch WEIDENREICH beitritt, glaubt, daß die Ausstoßung anzusehen ist als das Ergebnis von Veränderungen in der Oberflächenspannung zwischen Plasma und Zellkörper, Zellkörper und Kern. HOWELL (23) denkt an die Möglichkeit einer Auswanderung des Kerns als eines aktiven Prozesses.

Eine ausführliche Besprechung der verschiedenen Theorien und der Literatur findet man in den Veröffentlichungen von ISRAEL und PAPPENHEIM (24), BLOCK (2) und WEIDENREICH (55).

d) Myeloide Metaplasie der Milz.

Eine myeloide Metaplasie der Milz oder das Auftreten der verschiedenen Elemente des Knochenmarks — Myelocyten, Erythroblasten und Megakaryocyten — ist im Lauf der letzten Jahre unter den verschiedenartigsten Umständen gefunden worden: bei perniciöser Anämie, myeloider Leukämie, bei schweren sekundären Anämien (sowohl beim Menschen, wie auch bei Versuchstieren), bei schweren Infektionskrankheiten, bei Tumoren des Knochenmarks und Osteosklerose, und kürzlich auch bei lymphatischer Leukämie (HIRSCHFELD (21)).

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Zuerst herrschte die Ansicht vor, daß das Auftreten der genannten Elemente in der Milz auf einer Einwanderung und Weiterentwicklung unreifer Zellen aus dem Knochenmark beruhe. Zweifel an dieser Auffassung ergaben sich, als man Fälle beobachtete, bei welchen myeloide Elemente in der Milz gefunden wurden, ohne daß entsprechende Elemente im zirkulierenden Blut auftraten, und weiter, als man gelegentlich ähnliche Zellen in der normalen Milz vorfand (DOMINICI (12, 13), STERNBERG (50), KURPJUWEIT). Dies führte zu der Theorie einer autochthonen Entwicklung der myeloiden Elemente, sei es aus schon vorhandenen Zellen desselben Typus, sei es durch weitere Differenzierung einer Mutterzelle (des großen Lymphocyten) in der Milzpulpa. Die Mehrzahl der neueren Autoren hat sich der Annahme eines autochthonen Ursprungs des myeloiden Gewebes in der Milz angeschlossen (MEYER und HEINEKE (34, 35), MORRIS (37), DOMARUS (11), BUTTERFIELD (7), SCHRIDDE (46)); andere (STERNBERG (49), MORAWITZ und REHN (36)) betrachten beide Prozesse als möglich.

MEYER und HEINEKE wiesen nachdrücklich hin auf die Beziehungen des myeloiden Gewebes zum lymphoiden Gewebe hinsichtlich der Verteilung der verschiedenen Elemente. Das myeloide Gewebe entwickelt sich stets in der roten Pulpa (Sinus und Pulpastränge) und nicht in der weißen Pulpa (MALPIGHI'sche Körperchen); in dem Maße, wie es an Masse zunimmt, beeinträchtigt es die MALPIGHI'schen Körperchen und verursacht eine Druckatrophie derselben. MEYER und HEINEKE nehmen ebenso wie DOMARUS an, daß die Veränderungen in der Milz eine Rückkehr zum embryonalen Typus darstellen; daß die Milz ihre embryonale, hämatopoetische Funktion wieder aufnimmt, und daß es sich um einen kompensatorischen Prozeß handelt.

STERNBERG (49) gibt zwar die Möglichkeit eines autochthonen Ursprungs der myeloiden Zellen zu, hält aber eine Einwanderung von Knochenmarkselementen für wahrscheinlicher und bringt die myeloide Metaplasie lieber mit der Funktion der Milz als Blutfilter, als mit ihrer Funktion als hämatopoetisches Organ in Verbindung. Er findet, daß in vielen Fällen die Menge des anwesenden myeloiden Gewebes zu klein ist, als daß der Prozeß als myeloide Metaplasie aufgefaßt werden, oder von irgendwelchem kompensatorischen Wert sein könnte.

B. Benzolvergiftung.

In der großen Mehrzahl der Fälle, über die berichtet worden ist, handelt es sich um verhältnismäßig große Mengen des Giftes, die entweder per os, oder durch die Atmungsorgane aufgenommen wurden. Der Verlauf der Fälle, gleichgültig ob sie tödlich oder mit Genesung endeten, war ein akuter, und die Symptome stammten hauptsächlich vom Zentralnervensystem. Es ist demnach entweder die Zeit für die Entwicklung leukotoxischer Wirkungen zu kurz gewesen, oder, wenn solche Veränderungen aufgetreten sind, so sind sie übersehen worden.

SANTESSON (44) hat über eine Reihe von Fällen berichtet, bei denen als Folge einer fortgesetzt wiederholten Einatmung von Benzol — es handelte sich um das unreine, käufliche Produkt — eine chronische Benzolvergiftung auftrat; diese Fälle zeigten, ebenso wie die unsrigen, eine auffällige Verminderung der Zahl der weißen Blutzellen.

Die einzigen Angaben, die sonst noch in der Literatur über die Wirkung von Benzol auf das Blut und die hämatopoetischen Organe gemacht sind, finden sich in einer Experimentaluntersuchung von LANGLOIS und DESBOUIS (26, 27). Diese Beobachter fanden, daß die Dämpfe von

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Benzol und gewissen verwandten Kohlenwasserstoffen eine deutliche Zunahme der Zahl der roten Zellen im zirkulierenden Blut verursachten; eine Wirkung, die sie auf vermehrte Blutbildung zurückführten. Die Hyperglobulie verschwand zwei Tage nach dem Aussetzen des Einatmens der Dämpfe. Wurde das Gift subkutan injiziert, so wurde die Erscheinung nicht konstant beobachtet. Eine geringe Verminderung der weißen Blutzellen, welche gleichzeitig auftrat, wurde auf zufällige Ursachen zurückgeführt.

C. Einwirkung von Röntgenstrahlen auf das Blut und die hämatopoetischen Organe.

HEINEKE (18) hat gefunden, daß Röntgenbestrahlungen bei Kaninchen, Meerschweinchen und Mäusen eine ausgesprochene leukotoxische, insbesondere lymphotoxische Wirkung haben. Mit Rücksicht auf einige auffällige Analogien zwischen diesen Ergebnissen und der Wirkung von Benzolinjektionen soll seine Arbeit etwas näher betrachtet werden.

Die Veränderungen des lymphadenoiden Gewebes bestehen aus einer Zerstörung von Lymphocyten. Kurz nach Beginn des Zerstörungsprozesses erscheinen Phagocyten, welche Kernfragmente aufnehmen.

Die Lymphdrüsen werden auffällig arm an Zellen infolge der fast vollständigen Abwesenheit von Lymphocyten. Die Lymphfollikel des Darms und die MALPIGHI'schen Körperchen der Milz zeigen analoge Verhältnisse.

1. Milzpulpa.[1]) Ausgeprägte Veränderungen zeigen sich erst später als die oben angegebenen. Sie bestehen gleichfalls aus einer progressiven Zellzerstörung, für deren Zustandekommen eine größere Intensität und Dauer der Bestrahlung erforderlich ist. Der Pigmentgehalt der Milz nimmt in mäßigem Grade zu; die Zunahme ist proportional der Dauer des Lebens nach der Bestrahlung.

Im lymphadenoiden Gewebe und in der Milzpulpa sind die Veränderungen nach 12—14 Tagen geringer als nach 3—4 Tagen; eine Erscheinung, welche der Autor auf regenerative Vorgänge zurückführt.

2. Knochenmark. Gleichzeitig mit den Veränderungen in den lymphadenoiden Geweben erfolgt eine Zerstörung des Parenchyms des Knochenmarks. Die kleinen Lymphocyten und großen „nichtgranulären Myelocyten" (Myeloblasten — große Lymphocyten) leiden zuerst. Die neutrophilen, besonders die neutrophilen polynukleären Zellen sind am widerstandsfähigsten und herrschen überall vor. Nach 2—$2^1/_2$ Wochen beginnen regenerative Prozesse, die in 3—4 Wochen das Mark wieder zur Norm zurückführen. Die ungranulierten Formen (kleine und große Lymphocyten) regenerieren sich zuerst. Die kernhaltigen roten Zellen werden gleichzeitig mit den weißen Zellen des Marks zerstört.

3. Blut. Nach zwei oder mehr Tagen, je nach der Dauer der Bestrahlung, beginnen die weißen Blutzellen bis auf ca. 1000 im ccm abzunehmen. Qualitativ werden die Lymphocyten reduziert. Polynukleäre und große mononukleäre Elemente werden kaum betroffen. Abnorme Blutzellen werden nicht gefunden. Das Vorherrschen von polynukleären Zellen im Blut entspricht der Tatsache, daß der lymphatische Apparat in höherem Maße betroffen wird als die myeloiden Organe. Die roten Blutzellen zeigen bis zur dritten Woche keine Veränderung; erst dann ist ihre Zahl etwas vermindert.

HELBER und LINSER (20) zeigten, daß die Leukocyten unter der Einwirkung der Röntgenstrahlen im zirkulierenden Blut zerstört wurden.

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[1]) In seiner ersten Arbeit bespricht HEINEKE (18) das Knochenmark zusammen mit der Milzpulpa. Spätere Versuche (19) änderten die Auffassung und es wird demgemäß das Knochenmark für sich allein betrachtet.

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II. Material und Methoden.

Als Versuchstiere dienten für die meisten Untersuchungen ausgewachsene Kaninchen. Da Versuche, eine chronische Vergiftung durch Einatmung herbeizuführen, nicht zum Ziele führten, so wurden subkutane Injektionen angewandt. Reines Benzol (C_6H_6) wurde zwecks Abschwächung seiner reizenden Eigenschaften mit der gleichen Menge Olivenöl vermischt und das Gemisch unter den gebräuchlichen aseptischen Kautelen unter die Haut des Abdomens injiziert. Da hierdurch ein beträchtliches, über eine Fläche von 3—6 qcm sich erstreckendes Ödem erzeugt wurde, so wurde eine nachfolgende Injektion stets an einer anderen Stelle ausgeführt. Die Absorption des Benzol-Ölgemisches erfolgte langsam, und noch nach mehreren Tagen trat bei Inzision an der ödematösen Stelle ein ausgesprochener Geruch nach Benzol auf. Zuweilen wurden diese Stellen der Sitz eines chronischen entzündlichen Prozesses, welcher mit der Erzeugung einer Zone dichter Induration endete.

Die Injektionen wurden täglich ausgeführt; die Einzeldosis betrug 1 ccm pro kg Körpergewicht. Der Übersichtlichkeit halber sollen die Versuche, in drei Gruppen getrennt, besprochen werden.

1. Absteigende Reihe: neun Experimente, beginnend mit einem Tier, das 24 Stunden nach der ersten, und endend mit einem solchen, das 24 Stunden nach der neunten Injektion getötet wurde.

2. Sechs Versuche, bei denen die Tiere 5—10 Dosen erhielten und 2—5 Tage nach der letzten Injektion starben.

3. Aufsteigende Reihe. Zwölf Versuche. Es wurde der Versuch gemacht, eine Aplasie der hämatopoetischen Organe, besonders des Knochenmarks, herbeizuführen; danach sollte während wechselnden Zeitperioden Regeneration eintreten. Bei dieser Versuchsreihe ergaben sich beträchtliche Schwierigkeiten. Infolge von Unterschieden in der Empfindlichkeit der Tiere gegenüber dem Gift hatte die gleiche Zahl von Dosen durchaus nicht immer den gleichen Grad der Schädigung zur Folge. Es zeigte sich indes, daß die Zahl der weißen Blutzellen einen brauchbaren Index des Zustandes des Knochenmarks darstellte; ihre Zahl sank um so tiefer, je ausgeprägter die Aplasie wurde. Indem also der Kontrolle halber stets Leukocytenzählungen ausgeführt wurden, wurde so oft injiziert, daß die Zahl der Leukocyten auf 200—800 pro ccm sank. Auf diese Weise wurde in den meisten Fällen eine ausgeprägte Aplasie des Marks erzeugt. Wurde, wenn dieser Punkt erreicht war, noch ein- oder zweimal injiziert, so hatte dies gewöhnlich den Tod des Tieres zur Folge. Die Zahl der Injektionen schwankte zwischen vier und neun.

Der Kontrolle halber wurden vor Beginn der Injektionen 2 oder 3 Tage hindurch täglich von jedem Tier die roten und weißen Blutkörperchen gezählt und Blutabstrichpräparate gemacht. Bei vielen Versuchen wurden diese täglichen Zählungen die ganze Untersuchung hindurch fortgesetzt. Die Zahlen für die roten Blutkörperchen normaler Tiere schwankten zwischen 4 500 000 und 7 120 000 in ccm; die für die weißen zwischen 3000 und 11 680 im ccm. Es stimmt dies überein mit GRUBER'S (4) Befunden, nach denen die Zahl der roten Blutkörperchen beim Kaninchen normalerweise 4 500 000—7 500 000, die der weißen 5000—14 000 im ccm beträgt.

Die normalen Zählungen ergaben im allgemeinen ziemlich konstante Zahlen. Besonders gilt dies von den roten Blutzellen, bei denen Schwankungen von über 200 000 ungewöhnlich waren. Die weißen Zellen zeigten größere physiologische Schwankungen; wo diese aber so groß waren, daß sie eine klare Deutung des Versuchs nicht zuließen, wurde das Tier ausgeschaltet

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oder die Zählungen wurden fortgesetzt, bis sie an drei aufeinanderfolgenden Tagen annähernd konstant blieben.

In den meisten Fällen wurde die Sektion gleich nach dem Tode des Tieres ausgeführt. Das Material für die mikroskopische Untersuchung wurde in ZENKER'scher Lösung fixiert und mit Hämatoxylin und Eosin gefärbt. Das Knochenmark (Femur) wurde teilweise in ZENKER'scher Lösung fixiert und mit Hämatoxylin und Eosin oder Eosin-Orange-Toluidin (DOMINICI)[1]) gefärbt, teilweise wurde es in Sublimat-Alkohol fixiert und nach GIEMSA (15) gefärbt. Die Einbettung geschah stets in Paraffin. Knochenmarkstrichpräparate, von Femur und Rippen wurden mit Giemsalösung, Hämatoxylin-Eosin und mit Methylgrün-Pyronin gefärbt.

Nach den ersten zwei oder drei Injektionen blieben die Tiere meist in gutem Zustande. Danach wurden sie schwerfällig, zeigten Mangel an Freßlust und nahmen an Gewicht ab. In vorgeschrittneren Stadien der Vergiftung bestand Abmagerung, größere oder geringere Unsicherheit des Ganges, Tremor; schließlich zeigten sich Paralyse der Extremitäten, klonische und tonische Krämpfe, und endlich erfolgte der Tod. War die Vergiftung weniger weit vorgeschritten, so trat völlige Genesung ein.

III. Veränderungen im zirkulierenden Blute.

A. Allgemeines.

Der Verlauf der Veränderungen im Blut ergibt sich aus den nachstehend aufgeführten Tabellen. Aus jeder der drei Versuchsgruppen ist ein Beispiel angeführt.

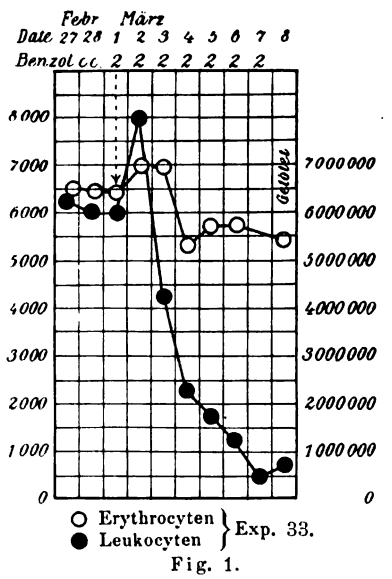


Fig. 1.

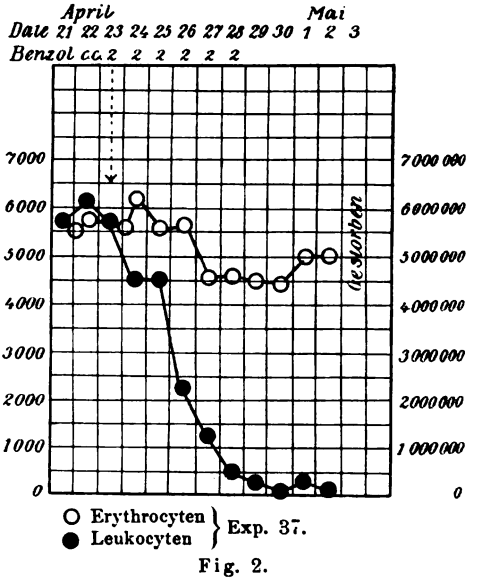


Fig. 2.

Versuch 33, Reihe I (Fig. 1). Ausgewachsenes Kaninchen. Gewicht 1940 g. Je 2 ccm Benzol in der gleichen Menge Olivenöl gelöst 9 Tage hindurch täglich injiziert.

Versuch 37, Reihe II (Fig. 2). Je 2 ccm Benzol mit der gleichen Menge Olivenöl 6 Tage hindurch täglich injiziert. Das Tier starb 3 Tage nach der letzten Injektion.

[1]) S. MAXIMOW (30).

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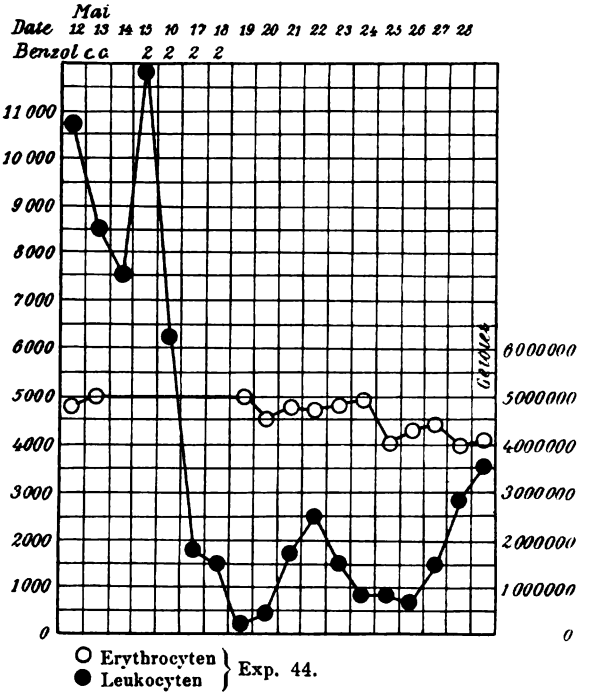


Fig. 3.

Versuch 44, Reihe III (Fig. 3). Ausgewachsenes Kaninchen, 1930 g. Je 2 ccm Benzol und Olivenöl 4 Tage hindurch täglich injiziert. Das Tier wurde 11 Tage nach der letzten Injektion getötet. Es befand sich andauernd in ausgezeichnetem Zustande.

B. Weiße Blutzellen.

Reihe I (absteigend).

In zwei Drittel der Fälle begannen die weißen Blutzellen innerhalb 24 Stunden nach der ersten Injektion an Zahl abzunehmen; die Abnahme betrug 80—6800 Zellen pro ccm; im Durchschnitt 2200. In einem Drittel der Fälle erfolgte innerhalb der ersten 24 Stunden eine Zunahme der weißen Blutzellen; sie betrug 240—2200 Zellen pro ccm, im Durchschnitt 1160. In allen Fällen war 24 Stunden nach der zweiten Injektion ein ausgesprochener Rückgang der Zahl der Leukocyten festzustellen. In dem Maße wie die Injektionen fortgesetzt wurden, erfolgte eine von Tag zu Tag weiter fortschreitende Abnahme der Leukocytenzahl. Nur gelegentlich trat einmal eine Unterbrechung des nach unten gerichteten Verlaufs der Kurve ein. In den schwersten Fällen wurden überhaupt keine Zellen mehr im graduierten Teil der Zählkammer gefunden und nur gelegentlich eine am Rand der Scheibe (s. Fig. 1 u. 2).

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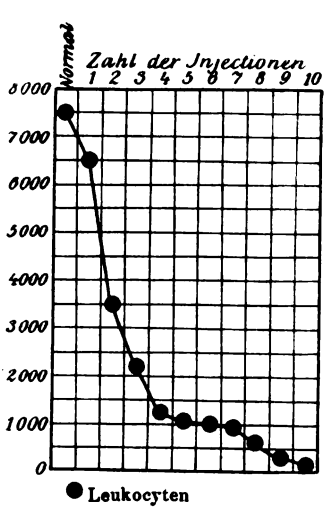


Fig. 4.

Durchschnittlicher Verlust an Leukocyten von Tag zu Tag während der Injektion.

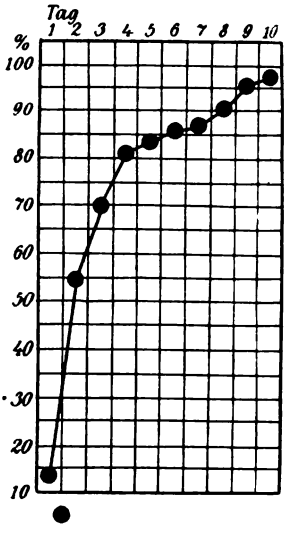


Fig. 5.

Prozentuale Abnahme der Leukocyten von Tag zu Tag während der Injektionen.

Fig. 4 gibt die durchschnittliche tägliche Zahl der Leukocyten, während der Injektionen.

In Fig. 5 ist unter Zugrundelegung der Durchschnittszahlen der prozentuale Zellverlust von Tag zu Tag angegeben.

Man sieht, daß 24 Stunden nach der zweiten Injektion über 50 % und 24 Stunden nach der dritten Injektion fast 75 % der weißen Blutzellen aus dem zirkulierenden Blut verschwunden sind. Nach der vierten Injektion wird die relative Abnahme weniger ausgeprägt.

Reihe II (tödlich endende Fälle).

In diesen Fällen ergaben die Zählungen nach Beendigung der Injektionen eine Herabsetzung der weißen Blutzellen bis auf 40—220 im ccm. Im weiteren Verlauf erfolgte eine noch weitere Abnahme oder aber die Zahl blieb bis zum Tode annähernd konstant; der Tod erfolgte zwischen dem ersten und siebenten Tage.

Reihe III (aufsteigend).

In der aufsteigenden Reihe ergaben sich beträchtliche Schwankungen zwischen den einzelnen Versuchsergebnissen. Der allgemeinste Typus war der, daß nach Beendigung der Injektionen die Zahl der weißen Blutzellen zunächst anstieg, dann absank und dann wiederum stieg und zwar so, daß langsam, aber stetig, die normale Zahl wieder erreicht wurde. Fig. 3 illustriert diesen Verlauf. In anderen Fällen blieb aber die Zahl mehrere Tage hindurch sehr niedrig und wieder in anderen stieg sie von Anfang an außerordentlich rasch. Zweifellos hängen diese Verschiedenheiten von zwei Faktoren ab; erstens von der Geschwindigkeit der regenerativen Veränderungen in den hämatopoetischen Organen und zweitens von der Menge des

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im zirkulierenden Blut noch vorhandenen Giftes. Infolge der Langsamkeit, mit welcher das Gift absorbiert wurde, war der zweite Faktor wahrscheinlich großen Schwankungen unterworfen.

Differentialzählungen.

Die Ergebnisse der Differentialzählungen sind für zwei Fälle mitgeteilt, für einen der absteigenden Reihe (Versuch 35) und für einen der aufsteigenden Reihe (Versuch 44). Wenn die Zahl der weißen Blutzellen größer war als 1000 im ccm, wurden im allgemeinen 250 Zellen gezählt; betrug ihre Zahl weniger als 1000 im ccm, so wurden 100 Zellen gezählt. Stets ist auch das Ergebnis der normalen Differentialzählung (vor Beginn der Injektionen) angegeben.

Versuch 35: Tötung 24 Stunden nach der achten Injektion.

Zahl der Injektionen	0	2	3	4	5	6
Polynukleäre Amphophile	44,0	58,0	54,0	36,0	1,0	9,0
Polynukleäre Eosinophile	2,4	2,0	1,0	0,0	0,0	0,0
Polynukleäre Basophile	0,4	0,8	0,0	0,0	0,0	0,0
Kleine Lymphocyten	43,2	32,4	35,0	61,0	86,0	74,0
Große Lymphocyten	0,4	0,8	1,0	0,0	1,0	8,0
Große Mononukleäre und Übergangsformen	8,8	5,2	8,0	0,0	10,0	5,0
Nicht rubriziert	0,8	0,8	1,0	1,0	2,0	4,0
Weiße Zellen im ccm	7000	3800	1560	1000	360	840

Versuch 44: Es sind die Resultate der nach Beendigung der Injektionen ausgeführten Zählungen angegeben. Nur vier Injektionen wurden in diesem Fall ausgeführt.

Erholungstag	Normal	1	2	3	4	5	6	8	9	10	11
Weiße Blutzellen im ccm	9000	280	440	1880	2480	1520	840	720	1720	2800	3400
Polynukleäre Amphophile	54,0	25,0	15,0	53,2	82,0	48,0	43,5	8,0	24,0	40,0	42,0
Polynukleäre Eosinophile	4,6	0,0	0,0	0,4	0,4	0,0	0,5	0,0	0,0	0,8	0,0
Polynukleäre Basophile	3,7	0,0	0,0	0,0	0,0	0,0	1,0	1,0	1,2	0,0	1,6
Kleine Lymphocyten	33,0	68,0	76,0	38,0	14,8	43,6	47,0	74,0	62,4	53,2	47,2
Große Lymphocyten	0,7	1,0	4,0	1,6	1,2	1,2	1,5	0,0	1,6	1,2	1,6
Große Mononukleäre Übergangsformen	3,6	5,0	2,0	4,0	0,4	3,2	5,0	15,0	10,0	4,8	8,0
Nicht rubriziert	0,4	1,0	3,0	2,8	0,4	3,6	1,0	2,0	0,8	0,0	0,0
Amphophile Myelocyten	0,0	0,0	0,0	0,0	0,8	0,4	0,5	0,0	0,0	0,0	0,0
Normoblasten	0	0	0	0	1	0	0	0	1	3	0

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Eine vor allem zu beachtende Tatsache ist die, daß die polynukleären Elemente relativ stärker betroffen sind als die Lymphocyten. Bei Versuch 35 findet man anfänglich eine relative Zunahme des Polynukleären, der jedoch eine außerordentlich rasche Abnahme folgt. Während der Erholung (Versuch 44) sind die Beziehungen wechseld, doch lassen die polynukleären Amphophilen und die kleinen Lymphocyten die Tendenz erkennen, wieder in relativ normaler Zahl aufzutreten. Die für die Normoblasten angegebenen Zahlen beziehen sich auf die Zählung von 250 weißen Blutzellen.

Viele der weißen Blutzellen, sowohl vom polynukleären wie vom mononukleären Typus, zeigen vorgeschrittene degenerative Veränderungen. Sie äußern sich besonders in einer partiellen Auflösung des Chromatins und einer daraus resultierenden ausgesprochenen Blässe und Deformierung des Kerns.

C. Rote Blutzellen.

In einem kleinen Teil der Fälle stieg die Zahl der roten Blutzellen nach der ersten Injektion an. Die zweite Injektion verursachte zuweilen eine noch stärkere Zunahme derselben; die dritte Injektion bewirkte aber stets einen Rückgang der Zahl der Erythrocyten auf ihren ursprünglichen Stand oder noch unter derselben herunter. LANGLOIS u. DESBOUIS (p. 687) erzielten durch Einatmenlassen von Benzol eine vorübergehende Polyglobulie; die bei subkutaner Injektion von ihnen erhaltenen Resultate sind aber nicht konstant.

Das Verhalten der roten Blutzellen ergibt sich aus den verschiedenen Tabellen. In der Mehrzahl der Fälle begann ihre Zahl nach der zweiten oder dritten Injektion abzunehmen; doch zeigen die einzelnen Fälle bedeutende Unterschiede. Zuweilen trat der Rückgang erst nach der vierten oder fünften Injektion auf und in zwei Fällen blieb die Zahl während der ganzen Versuchszeit so gut wie konstant.

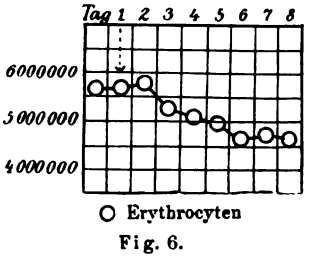


Fig. 6.
Durchschnittszahlen der Erythrocyten während der Infektionen.

Fig. 6 gibt die täglichen Durchschnittszählungen während der Injektionszeit wieder.

Man sieht, daß die Verminderung der Zahl der Erythrocyten der der Zahl der Leukocyten keineswegs vergleichbar ist. Erstere haben nach Verlauf von 8 Tagen 16 %, letztere 92 % ihres ursprünglichen Betrages verloren.

Während der Erholungsperiode (Reihe III) blieb die Zahl der roten Blutkörperchen annähernd konstant. In einigen Fällen zeigte sich eine unbedeutende weitere Abnahme, in vielen Fällen keine Veränderung. Bemerkenswert ist, daß die roten Zellen im Gegensatz zu den weißen keine Tendenz zeigten zur Norm zurückzukehren (s. Fig. 3 u. 4).

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D. Zusammenfassung.

Die Verabfolgung von Benzol hat ein rasches Verschwinden der weißen Zellen aus der peripheren Zirkulation zur Folge, wobei zuweilen ein anfängliches Steigen ihrer Zahl vorhergeht. In den am meisten vorgeschrittenen Fällen sind so gut wie keine weißen Blutzellen mehr vorhanden.

Zur Erklärung der Erscheinung kann man annehmen entweder, daß die Leukocyten sich in anderen Teilen der Zirkulation ansammeln, oder daß sie absterben. Die erste Möglichkeit ist deshalb ausgeschlossen, weil in den Blutgefäßen des ganzen Körpers weiße Blutzellen abwesend sind. Die Zellen sind demnach zerstört worden, und es erhebt sich die Frage, ob die Zerstörung einer Abtötung der Zellen durch das Gift zuzuschreiben ist, oder ob es sich um natürlichen Zelltod, verbunden mit einem Mangel an Regeneration handelt. Wie weiter gezeigt werden soll, leiden die hämatopoetischen Organe derartig, daß sie zu einer wirksamen Wiedererzeugung von Zellen, solange das Gift verabfolgt wird, unfähig sind.

Über die natürliche Lebensdauer der weißen Blutzellen ist wenig bekannt. Nakanishi [39]) hat unter Anwendung einer Methode der vitalen Färbung gefunden, daß, wenn Blut aus dem Körper entfernt und 2 Wochen lang auf Eis gehalten wurde, ein wesentlicher Teil der Leukocyten lebensfähig blieb und beim Erwärmen amöboide Bewegungen zeigte. Nach dieser Beobachtung ist es sehr unwahrscheinlich, daß, selbst in Abwesenheit regenerativer Veränderungen, 75 % der Leukocyten in 3 Tagen sterben sollten. Ferner zeigt die Untersuchung von Blutabstrichpräparaten oft degenerierende Formen unter den weißen Blutzellen, besonders unter den polymorphonukleären Leukocyten.

Diese Tatsachen rechtfertigen die Schlußfolgerung, daß das Gift die Leukocyten im zirkulierenden Blut in großer Ausdehnung abtötet. Helber und Linser [20]) haben gezeigt, daß das gleiche zutrifft bei Tieren, die der Einwirkung von Röntgenstrahlen ausgesetzt waren.

Differentialzählungen ergeben eine stärkere Reduktion der Zahl der polymorphonukleären als der der mononukleären Elemente. Dies entspricht der Tatsache, daß das Knochenmark schwerer geschädigt wird als die lymphadenoiden Organe. Nach Bestrahlung mit Röntgenstrahlen (Einleitung, p. 683) leiden die Lymphocyten mehr als die polymorphonukleären Zellen, und hier sind die lymphadenoiden Organe stärker betroffen als das Knochenmark.

Die roten Blutzellen werden in viel geringerem Grade angegriffen als die weißen. In einigen Fällen ergab die Zählung von Anfang bis zu Ende des Versuchs so gut wie konstante Resultate,

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und der Durchschnitt aller Fälle zeigt einen Verlust von nur 12% während der ersten Woche. Ein derartiger Rückgang ist so geringfügig, daß zu seiner Erklärung weitere Momente als der natürliche Zelltod[1]) in Abwesenheit von Regeneration nicht herangezogen zu werden brauchen. In einigen wenigen Fällen zeigt sich dagegen ein ausgesprochener Rückgang; vermutlich spielt hierbei die Gegenwart des Benzols in der Zirkulation doch eine Rolle. Die Zunahme des Pigmentgehaltes der Milz ist nicht sehr groß; sie läßt sich, wenigstens teilweise, auf die ausgedehnte Zerstörung kernhaltiger roter Zellen im Mark zurückführen. HEINEKE erhielt nach Bestrahlung mit Röntgenstrahlen ähnliche Resultate. In beiden Versuchsreihen ergaben sich nur geringe Veränderungen der roten Blutzellen trotz vorgeschrittener Aplasie des Marks. Dies spricht für die Richtigkeit der Annahme, daß die natürliche Lebensdauer der Erythrocyten eine relativ lange ist.

Nach Beendigung der Injektionen bleibt die Zahl der roten Blutzellen im allgemeinen annähernd konstant. Sie zeigen eine viel geringere Tendenz zur Norm zurückzukehren, als die weißen. Im Gegensatz hierzu sind die Erythroblasten während der Frühstadien der Regeneration oft viel reichlicher im Knochenmark vertreten als die Granulocyten.

IV. Knochenmark.

A. Degenerative Veränderungen.

Versuch 29; Tötung 24 Stunden nach der ersten Injektion.

Bei schwacher Vergrößerung erscheint das Knochenmark annähernd normal, d. h. eine ausgedehnte zentrale Zone um den Zentralsinus herum wird fast vollständig von Fettzellen eingenommen, wogegen die Elemente des Parenchyms auf die mehr peripheren Teile der Schnitte beschränkt sind. In diesen peripheren Gebieten haben die zellulären Elemente eine deutlich ausgeprägte Zunahme ihrer Zahl erfahren. Bei starker Vergrößerung sieht man, daß die Zunahme der Parenchymzellen — die Hyperplasie — alle Elemente ziemlich gleichmäßig betrifft. Große Lymphocyten und Megaloblasten sind nicht zahlreich.

Ueberall zeigen die Elemente des Parenchyms reichlich mitotische Figuren, das Zeichen einer aktiven Proliferation. Bisher zeigt nur ein geringer Bruchteil der Zellen Zeichen einer Schädigung.

Versuch 28; Tötung 24 Stunden nach der zweiten Injektion.

Die Hyperplasie ist weiter vorgeschritten als im vorhergehenden Fall, so daß jetzt das gesamte Mark von der Peripherie bis zum Zentralsinus, cellulär ist (Fig. IV.) Die Zellen liegen in dichten, anastomosierenden Massen; sie sind voneinander getrennt durch Gruppen von Fettzellen, durch

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[1]) GRAWITZ (16) gibt an, daß nach allgemeiner Auffassung das Leben einer roten Blutzelle 3—4 Wochen dauert. Dies würde einen Verlust von 25—33% der roten Zellen in einer Woche erklären.

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dilatierte und verschlungene Kapillaren und durch ödematöses Reticulum. Der Typus der Hyperplasie ist ähnlich dem, wie er im letzten Abschnitt beschrieben wurde.

Viele der Parenchymzellen zeigen Anzeichen von Karyolyse und Karyorrhexis. In einigen sind die Kerne blaß, und das Chromatin ist zum Teil aufgelöst; in anderen bildet das Chromatin zerstückelte Klumpen und Massen. Ähnliche Chromatinmassen finden sich frei im Gewebe oder in den Leibern von Phagocyten angesammelt. Ein beträchtlicher Teil der Myelocyten, sowohl der amphophilen wie der eosinophilen, und der großen Lymphocyten zeigt diese degenerativen Veränderungen. Amphophile Myelocyten sind die vorherrschenden Zellen.

Polymorphonukleäre Leukocyten, sowohl amphophile wie eosinophile, sind in relativ viel geringerer Zahl vorhanden als bei dem vorhergehenden Versuch. In einigen derselben sieht man degenerative Erscheinungen ähnlich denen, wie sie für die Myelocyten beschrieben wurden, aber die große Mehrzahl sieht normal aus.

Die kernhaltigen roten Zellen bleiben von den durch das Gift bewirkten Veränderungen nicht verschont. In vielen sind die Kerne deformiert, geschrumpft, pyknotisch und unregelmäßig; in anderen zeigt sich eine partielle Auflösung des Chromatins.

Die Megakaryocyten sind der allgemeinen Hyperplasie entsprechend vermehrt, aber viele von ihnen zeigen vorgeschrittene Grade von Karyolyse und Karyorrhexis. Sie zeigen eine ausgesprochene phagocytische Tätigkeit, indem sie in ihrem Cytoplasma einen oder mehrere polynukleäre Leukocyten in verschiedenen Stadien der Verdauung einschließen. Oft ist die eingeschlossene Zelle von einer runden, hellen, sich nicht färbenden Zone umgeben.

Mitosen sind nicht so reichlich wie bei dem vorhergehenden Versuch.

Außer den beschriebenen Zellen finden sich überall verstreut, am reichlichsten vielleicht an der Peripherie, Zellen mit einem dunkeln, runden, ziemlich tief gefärbten Kern von ungefähr der Größe des Kerns eines kleinen Lymphocyten und mit verhältnismäßig dunkel gefärbtem Protoplasma. Das Protoplasma zeigt oft eine hellere Zone neben dem Kern und weißt kurze stumpfartige Fortsätze auf. Zweifellos gehören diese Zellen zur Gruppe der Polyblasten MAXIMOW (29). Pigmenthaltige Zellen sind in geringer Zahl auch vorhanden.

Versuch 26; Tötung 24 Stunden nach der fünften Injektion.

Als ein Zeichen hyperplastischer Veränderungen treten Elemente des Parenchyms nicht nur in der Peripherie, sondern im ganzen Schnitt auf. Die Zerstörung äußert sich darin, daß die Zellen, statt in dichten Gruppen und Massen zusammenzuliegen, nur in kleinen gerissenen Ketten und Bändern, in Gruppen von zweien oder dreien, oder ganz isoliert angetroffen werden. Zwischen den Gruppen liegen relativ große Mengen ödematösen Reticulums und viele Fettzellen. Der Gesamtgehalt des Marks an Zellen ist subnormal (Fig. II).

Alle Zelltypen sind an den degenerativen Veränderungen beteiligt. Amphophile Myelocyten herrschen noch vor. Die polynukleären Amphophilen zeigen, obwohl geringer an Zahl, doch einen geringeren Prozentsatz an degenerativen Formen. Eosinophile Zellen, myelocytische und polynukleäre, sind sehr spärlich geworden. Kernhaltige rote Zellen sind nicht zahlreich. Meistenteils finden sie sich einzeln oder in kleinen, versprengten, unregelmäßigen Gruppen, und viele von denen, die noch zurückbleiben, sind

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degeneriert. Die Zahl der Megakaryocyten ist stark reduziert und nur wenige normal aussehende Exemplare sind noch zu finden. Mitotische Figuren sind verhältnismäßig selten, und viele derselben sind anormal.

Neben diesen Zelltypen finden sich Zellen, die den kleinen Lymphocyten des Blutes gleichen und die in Zukunft lediglich als kleine Lymphocyten bezeichnet werden; auch andere, die den im letzten Abschnitt beschriebenen Polyblasten ähnlich sind. Diese Zellen scheinen keine Tendenz zu haben, in Gruppen aufzutreten; sie liegen isoliert, voneinander getrennt, und oft völlig unabhängig von allen Elementen des Parenchyms. Phagocyten, welche Kerndetritus und Pigmentkörner enthalten, sind in spärlicher Zahl vorhanden.

Versuch 33; Tötung 24 Stunden nach der siebenten Injektion.

Der Schnitt zeigt viel ausgesprochenere Veränderungen als die vorhergehenden. Zerstörende Prozesse haben die proliferativen stark überwuchert, so daß nur noch sehr wenig spezifische Knochenmarkzellen existieren. Parenchymzellen findet man isoliert oder in Gruppen von zweien und dreien unter Bildung relativ großer Zwischenräume in dem ödematösen Reticulum verstreut. Von diesen Elementen sind die polynukleären Amphophilen am reichlichsten vorhanden. Ein großer Teil derselben zeigt jetzt Anzeichen der Degeneration. Amphophile Myelocyten sind in geringer Zahl und kernhaltige rote Zellen nur in kleinen, weit versprengten Gruppen vorhanden. Einige wenige Megakaryocyten bestehen noch, aber in mehr oder weniger degenerierter Form. Große Lymphocyten und eosinophile Zellen sind so gut wie abwesend.

Kleine Lymphocyten und Polyblasten sind auch hier wie in den vorhergehenden Schnitten, anwesend. Phagocyten mit braungelbem Pigment und Kernfragmenten sind ziemlich reichlich zugegen.

Versuch 39: neun Injektionen.

Das Tier starb wenige Stunden nach der letzten Injektion. Dieser Versuch kann als typisch angesehen werden für den Verlauf einer großen Anzahl von Fällen, in denen die Tiere entweder starben oder in dem gleichen Stadium getötet wurden.

Das Mark zeigt fast absolute Aplasie (Fig. III). Im Querschnitt des Oberschenkelmarks findet man nur 1—2 Parenchymzellen und diese sind stets degeneriert. Die Blutgefäße sind dilatiert und enthalten normal aussehende Erythrocyten, gelegentlich einen Lymphocyten und keine polynukleären Zellen. Das Reticulum ist ödematös, die Fettzellen sind gering an Zahl und geschrumpft. Die Kerne der Bindegewebszellen und Endothelzellen sind gut erhalten, wenngleich einige von ihnen geschwollen und blaß sind. Neben ihnen findet man Zellen, deren Auftreten schon in den früheren Schnitten bei partieller Aplasie konstant beobachtet wurde, nämlich:

1. Viele kleine Lymphocyten. Sie liegen in der Adventitiaschicht der Blutgefäße, in der Bindegewebskapsel des Marks und versprengt im Reticulum. Gewöhnlich sind sie isoliert; nur selten treten sie in Gruppen auf. Sie zeigen keinerlei Zeichen von Entartung.

2. Polyblasten. Die Mehrzahl derselben nähert sich dem Typus der Plasmazelle. Sie besitzen einen kleinen, exzentrisch gelagerten, chromatinreichen Kern von ungefähr der Größe des Kerns eines kleinen Lymphocyten. In einigen Zellen sieht man 2 kleine Kerne. Das Protoplasma ist dicht, deutlich basophil und läßt oft in der Nähe des Kerns eine heller gefärbte

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Zone erkennen. Solche Zellen sind birnförmig rund oder oval, oder sie besitzen kurze stumpfartige Fortsätze.

3. Pigmenthaltige Zellen. Sie sind relativ groß und besitzen einen sich leicht anfärbenden, bläschenförmigen Kern und reichliches Protoplasma. Sie können rund, oval oder spindelförmig sein, und können unregelmäßige Fortsätze besitzen. Im Cytoplasma sind viele braungelbe, grobe Pigmentkörner und gleichzeitig zuweilen andere Körner vorhanden, die eine hell bis dunkelblaue Färbung annehmen. Einige gelbe Pigmentmassen sind auch außerhalb der Zellen vorhanden.

Zusammenfassung: Der letzte Abschnitt zeigt das Endstadium der Vergiftung: Eine fast vollständige Aplasie des Marks. Sogar die Widerstandsfähigeren polynukleären Amphophilen sind verschwunden. Die einzigen Elemente, die neben den fixen Gewebszellen vertreten sind, sind kleine Lymphocyten und Polyblasten. Diese beiden Zelltypen wurden bei allen Versuchen der absteigenden Reihe gefunden, aber mit Rücksicht auf die Unsicherheit hinsichtlich ihres Ursprungs und ihrer Bedeutung sollen sie, von den Parenchymelementen gesondert, für sich besprochen werden.

Im allgemeinen nähern sich die Polyblasten dem Plasmazelltypus. Die kleinen Lymphocyten sind von denen des zirkulierenden Bluts in keiner Weise zu unterscheiden. Sie finden sich gelegentlich in Form dichter Gruppen in der Adventitialschicht der großen Arterien oder in der Umgebung eines kleinen Gefäßes. Viel häufiger aber kommen beide Zelltypen isoliert vor, entweder in der Bindegewebskapsel des Marks, oder im Reticulum verstreut. Sie sind voneinander getrennt und unabhängig von den Elementen des Parenchyms.

Es erhebt sich nun die Frage, woher diese Zellen stammen. Pappenheim (42), Dominici (13) u. A. geben an, daß Lymphocyten ein normaler Bestandteil des Marks seien, aber im Leben des Erwachsenen sind sie in sehr geringer Zahl zugegen. Gelegentlich finden sich auch Plasmazellen unter normalen Bedingungen im Mark.

1. Sind die Zellen, welche man in dem Schnitt, um den es sich hier handelt, sieht, normale Bestandteile des Marks, die durch Zerstörung der Parenchymelemente sichtbar gemacht sind? Der Hauptfaktor, welcher gegen diese Annahme spricht, ist das reichliche Auftreten von den Zellen beider Typen, die normalerweise im Knochenmark sehr spärlich vertreten sind.

2. Sind sie normale Bestandteile, die gewuchert sind? Das Studium der regenerativen Stadien wird zeigen, daß regenerierende Zellen im Knochenmark die Tendenz haben, sich insel- oder gruppenartig anzuordnen.

Sogar die polynukleären Leukocyten, welche bedeutende amöboide Fähigkeiten haben, zeigen in vielen Fällen diese Gruppierung. Die Lymphocyten, um die es sich hier handelt, treten aber nur gelegentlich in Form von Gruppen in der Umgebung eines Blutgefäßes auf; viel

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häufiger erscheinen sie als isolierte Exemplare, die im Reticulum verstreut sind.

3. Leiten sie sich von den Clasmatocyten ab? Die Betrachtung von Experiment (39), bei welcher die Parenchymelemente nahezu verschwunden sind, lehrt, daß Zellen, die als Clasmatocyten angesprochen werden könnten. in verhältnismäßig kleiner Zahl anwesend sind. Die vorhandenen zeigen keine Anzeichen von mitotischer Tätigkeit und keine besondere Verwandtschaft zu den Lymphocyten und Polyblasten.

4. Leiten sie sich ab von den Lymphocyten des zirkulierenden Blutes, wie es MAXIMOW für ähnliche Zellen unter chronisch entzündlichen Bedingungen beschreibt? Wie sich zeigen wird, wird das lymphadenoide Gewebe im ganzen Körper durch das Gift geschädigt, und viele der Zellen, welche dasselbe zusammensetzen, sowohl große wie kleine Lymphocyten werden getötet. Es wird aber weniger schwer betroffen als das myeloide Gewebe. In den späteren Stadien der Vergiftung. nachdem die Zahl aller Zellen des zirkulierenden Blutes reduziert ist, herrschen unter den noch bestehenden Zellen die Lymphocyten bei weitem vor. Auch in diesen Stadien können gelegentlich noch Lymphocyten gefunden werden, die durch die Wand einer der Knochenmarkskapillaren hindurchwandern.

Das Studium des vorliegenden Materials ergibt, daß die letzte der aufgeführten Möglichkeiten die wahrscheinlichste ist, wenngleich eine definitive Antwort auf die Frage noch nicht gegeben werden kann. Das interessante Ergebnis ist aber, daß bei der Benzolvergiftung die kleinen Lymphocyten und Polyblasten, was nun auch immer ihr Ursprung sein mag, sich als die widerstandsfähigsten aller Parenchymzellen erweisen. Sie bestehen und nehmen anscheinend an Zahl noch zu, nachdem bereits alle anderen Knochenmarkselemente verschwunden sind.[1])

B. Regenerative Veränderungen.

Für die Besprechung der im Mark auftretenden regenerativen Veränderungen ist es nicht möglich, die Versuche nach der Zahl der Injektionen oder nach der Zahl der der letzten Injektion folgenden Tage zu ordnen, da die Schwankungen in den einzelnen Fällen zu groß sind. Es können lediglich die verschiedenen Stadien beschrieben werden, wobei mit dem frühesten Stadium begonnen, und jedesmal die experimentellen Bedingungen mitgeteilt werden sollen.

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[1]) Im Anschluß an diese Ausführungen sei erwähnt, daß bei HEINEKE's Versuchen (s. Einl.) sowohl die großen, wie die kleinen Lymphocyten die ersten Zellen waren, welche aus dem Mark verschwanden.

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Versuch 38: zehn Injektionen. Der Tod erfolgte 72 Stunden nach der letzten Injektion.

Bei schwacher Vergrößerung unterscheidet sich das Mark nicht wesentlich von dem Bilde, das der vorhergehende Schnitt darbot. Die Wirkung des Giftes zeigt sich in Ödem, Hyperämie und Aplasie. In einigen Teilen des Schnittes ist die Aplasie so gut wie vollständig, in anderen, besonders in der Nähe der Peripherie, findet man Parenchymzellen. Die Zellen an der Peripherie treten weder einzeln auf, noch bilden sie unbestimmte, voneinander durch weite Zwischenräume getrennte Reihen von zweien oder dreien. Sie bilden vielmehr deutliche, scharf umschriebene Gruppen und Inseln, und erscheinen bei schwacher Vergrößerung als kleine, blaue Zonen, die von dem umgebenden rosa gefärbten, ödematösen Reticulum scharf abgegrenzt sind. Sie erinnern sofort an das Bild von Hämatopoese, welches in den Mesenchymgeweben des Embryos auftritt, wie es z. B. von SAXER (45), MAXIMOW (31), u. A. (s. Einl.) beschrieben ist.

Bei diesem Schnitt macht sich die Einwirkung des Giftes noch geltend und viele Zellen in den Inseln zeigen degenerative Veränderungen. Die Einzelheiten des regenerativen Prozesses sollen deshalb in den folgenden Abschnitten beschrieben werden.

Versuch 53: sieben Injektionen. Tötung 4 Tage nach der letzten Injektion; und

Versuch 50: fünf Injektionen. Tötung 6 Tage nach der letzten Injektion.

Die Schnitte lassen deutlich eine schwere Toxämie erkennen. Eine ausgedehnte Zellzerstörung hat stattgefunden, so daß noch jetzt, 4 resp. 6 Tage nach Beendigung der Injektionen große Flächen zurückgeblieben sind, in denen die Aplasie fast vollständig ist. Solche Flächen enthalten nur retikuläre und Endothelzellen, kleine Lymphocyten und Polyblasten. Die Kapillaren sind dilatiert und mit Blut prall gefüllt, die Fasern des Reticulums sind durch Ödem auseinandergezogen und die Fettzellen sind geschrumpft und deformiert.

Rings versprengt finden sich kleine, mehr oder weniger scharf umschriebene und gewöhnlich voneinander völlig isolierte Zellnester und Zellgruppen, welche zu keiner bestimmten Zone in erkennbarer Beziehung stehen. Wo zwei solche Gruppen nahe beieinander liegen, ist es gewöhnlich zu einer Vermischung der Zellen am Berührungsrand gekommen. (Fig. V und VI.)

Bei schwacher Vergrößerung lassen sich in diesen Zellnestern zwei Typen unterscheiden: einer, welcher Zellen mit kleinen dunklen Kernen enthält und aus kernhaltigen roten Zellen besteht und ein zweiter, welcher größere, blassere Zellen enthält und aus Granulocyten zusammengesetzt ist.

Die genannten Zellnester sind extravaskulär und viele befinden sich in unmittelbarer Berührung mit der Wand einer Kapillare oder einer dünnwandigen Vene. Die innersten Zellen liegen dem Endothel an und von hier aus breiten sich die Zellen entweder längs der Gefäßwand aus oder sie sind in senkrechter Richtung zu ihr angehäuft.

Erythroblastennester (Fig. XIX): es sind kleine, scharf umschriebene Inseln, die aus einer kompakten Gruppe von 3 oder 5 bis zu 12 oder 15

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Zellen bestehen. Man findet in ihnen alle Typen von Erythroblasten: die jüngeren Formen mit ihren relativ großen, blassen Kernen und spärlichem, basophilem Protoplasma; die typischen Normoblasten mit kleineren, tiefer gefärbten Kernen und reichlicherem Protoplasma, und ältere Zellen mit pyknotischen Kernen von runder oder von Klee- oder Rosettenform. Die Normoblasten sind die vorherrschenden Elemente, aber auch die jüngeren Formen sind ziemlich reichlich vertreten und zwar begleiten sie entweder die Normoblasten, oder sie bilden unabhängige Inseln.

Granulocyteninseln: Fig. XVIII. Im Gegensatz zu den Erythroblasteninseln stehen andere, deren Umrisse unregelmäßiger sind und deren Zellen weniger kompakt, aber größer sind und sich weniger tief anfärben. Es sind die Granulocytengruppen; sie bestehen aus großen Lymphocyten (Myeloblasten) Myelocyten, Metamyelocyten und Polynukleären. Myelocyten und Metamyelocyten herrschen vor, aber in den meisten Gruppen finden sich Myeloblasten und polynukleäre Elemente, die sich ihnen in wechselnden Mengenverhältnissen zugesellen. Unter den körnigen Zellen sind die amphophilen reichlicher vertreten als die eosinophilen, welch letztere äußerst spärlich sind. In einigen der Gruppen sind die Zellen einander ziemlich nahe angeordnet, in den meisten aber liegen sie etwas voneinander getrennt und kleine Zellbänder gehen von der Peripherie der Gruppe aus. Wo solche Nester nahe beieinander liegen, vereinigen sich die auswärts liegenden Fortsätze und bilden ein unregelmäßiges Netzwerk.

Nester von großen Lymphocyten: neben den Granulocyten — und Erythroblastennestern finden sich einige wenige, die lediglich aus großen Lymphocyten bestehen. Im allgemeinen enthalten sie nur wenige Zellen, etwa 3—8, und ihre Struktur ist eine fast ebenso kompakte wie die der Erythroblastenherde. Die Größe ihrer Komponenten variiert beträchtlich; sie zeigen einen verhältnismäßig großen, blassen Kern mit deutlichem Kernkörperchen, und geringe Mengen eines stark basophilen, nicht körnigen Protoplasmas.

Unregelmäßig zwischen diese Inseln zerstreut finden sich isolierte Zellen, welche die verschiedenen Zelltypen darstellen (Fig. XVIIId). Amphophile Myelocyten sind am reichlichsten vertreten, aber auch die großen Lymphocyten sind ziemlich zahlreich und gelegentlich findet sich ein großer Erythroblast. In all diesen Zellinseln und ebenso in den isolierten Zellen treten mitotische Figuren in großer Zahl auf. Wie in der absteigenden Reihe, so sind auch hier kleine Lymphocyten und Polyblasten überall als isolierte Exemplare zugegen.

Megakaryocyten: Auch die Riesenzellen zeigen deutliche Regeneration, wenngleich sie nicht in großer Zahl zugegen sind. Sie treten einzeln oder in Gruppen von 2—3 nahe aneinander gelagerten Zellen auf. Oft liegen mehrere solcher kleiner Gruppen beieinander und bilden eine große Insel von etwa 10—12 Zellen. Unter ihnen sieht man nicht nur den ausgewachsenen Typus mit seinem stark zusammen gerollten Kern und reichlichem Protoplasma, sondern auch jüngere Zellen, in denen der Kern noch rund und das Protoplasma verhältnismäßig spärlich ist. Zwischen ihnen und der voll entwickelten Zelle findet man alle Arten von Übergängen, wie sie z. B. von Saxer(45) beschrieben worden sind.

Phagocytenzellen mit hell- bis dunkelgelbem Pigment sind überall im Schnitt ziemlich reichlich vorhanden. Ein ähnliches Pigment findet sich in vielen Endothelzellen. In den Blutgefäßen sind weiße Blutzellen nicht zahlreich; die Lymphocyten sind relativ viel reichlicher vertreten als die Polynukleären.

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	Versuch 50.		Versuch 53.	
	Weiße Blutzellen	Rote Blutzellen	Weiße Blutzellen	Rote Blutzellen
Normal	8500	5 000 000	7000	4 650 000
Nach Beendigung der Injektion	800	4 280 000	880	4 600 000
Am Tage des Todes	1080	4 380 000	530	4 536 000

Zusammenfassung: Die Schnitte zeigen ein aplastisches Mark, in welchem kleine, umschriebene Zellgruppen und Zellinseln liegen. Sie zeigen drei verschiedene Typen, die aus Erythroblasten, Granulocyten und großen Lymphocyten bestehen.

Wenn auch in den Granulocyteninseln die Myelocyten (amphophile) und in den Erythroblasteninseln die Normoblasten vorherrschen, so sind doch auch die jüngeren Zelltypen einigermaßen zahlreich vertreten.

Ein Vergleich dieses Schnittes mit den nachstehend zu beschreibenden lehrt, daß es zweifellos frühe regenerative Veränderungen in einem Mark darstellt, welches eine vorgeschrittene Aplasie erfahren hat.

Versuch 51: acht Injektionen; Tötung 5 Tage nach der letzten Injektion.

Es besteht Hyperämie, deutliches Ödem und fast gänzliche Abwesenheit von Fettzellen. Der Zellgehalt des Marks ist erheblich unter die Norm heruntergegangen, ist aber viel größer als in den eben beschriebenen Schnitten 50 und 53. Die Zellen sind in der peripheren Hälfte des Schnittes angesammelt und lassen den zentralen Teil fast frei. Die Peripherie ist aber keineswegs gleichmäßig ausgefüllt. Segmente, die dicht mit proliferierenden Parenchymzellen infiltriert sind, wechseln ab mit solchen, die relativ arm sind an solchen Zellen; hier und da findet man auch ein Segment, in welchem fast völlige Aplasie besteht.

In den zellulären Zonen sieht man die Inselbildung in typischer Weise. Die meisten Inseln bestehen aus Erythroblasten. Sie sind viel größer als die früher beschriebenen; Gruppen von 20—30 sind das gewöhnliche, und wo diese großen Nester reichlich auftreten, verschmelzen sie miteinander zu einem unregelmäßigen, weitmaschigen Netzwerk. Die Zellen, welche die Erythroblasteninseln zusammensetzen, stellen die verschiedenartigsten Typen dar, von den großen jungen Formen mit bläschenförmigem Kern und relativ spärlichem basophilem Protoplasma bis zu den Normoblasten und den alten, pyknotischen Zellen, mit reichlichen, hämoglobinhaltigem Protoplasma. Die Normoblasten herrschen vor, aber auch die jüngeren Elemente sind noch sehr reichlich. Gelegentlich findet man einen isolierten Erythroblasten im Reticulum zwischen den Inseln. Granulocytische Gegenden sind viel seltener; nur in einem Teil des Schnittes ist eine große Gruppe dieser Zellen anzutreffen. Sie zeigt eine lockere, verzweigte Anordnung; ihre Ausläufer dringen in die Gebiete zwischen den Erythroblastennestern ein. Die Zellen, welche sie zusammensetzen, sind hauptsächlich amphophile Myelocyten und Metamyelo-

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cyten; darunter befinden sich noch beträchtliche Mengen großer Lymphocyten. Irgendwelche polynukleäre Zellen und eosinophile Myelocyten sind nur in sehr geringer Zahl anwesend. Neben diesem großen Herd findet man hier und dort kleinere Gruppen weißer Blutzellen, und zwischen ihnen liegen isolierte Zellen der verschiedenen Typen.

Megakaryocyten sind relativ sehr zahlreich. Sie treten in 4 oder 5 Gruppen auf, deren jede aus 5—10—12 Zellen besteht, hier und da auch als isolierte Exemplare. Die Entwicklungsstadien lassen sich, wie vorher, verfolgen und gelegentlich findet man mitotische Figuren. Kleine Lymphocyten findet man überall zerstreut, wogegen Polyblasten weniger reichlich auftreten als in den früheren Schnitten. Mitotische Figuren findet man überall in großer Menge.

	Weiße Blutzellen	Rote Blutzellen
Normal	9500	4 700 000
Nach Beendigung der Injektionen	640	3 745 000
Am Tage des Todes	1120	3 168 000

Zusammenfassung: Obwohl die Zahl der Injektionen größer und die für die Erholung eingeräumte Zeit ungefähr die gleiche war, wie bei den Versuchen 50 und 53, so sind doch die regenerativen Prozesse hier viel weiter vorgeschritten.

Zonen vollständiger Aplasie bestehen zwar noch, aber sie sind kleiner und geringer an Zahl als vorher. Parenchymelemente sind viel zahlreicher. Wenngleich die Inselbildung noch vorhanden ist, so sind doch die Inseln an Größe gewachsen, und es besteht größere Neigung zum Zusammenfließen aneinandergrenzender Gruppen. Amphophile Myelocyten und Normoblasten sind die in den Granulocyten- bzw. Erythroblasteninseln vorherrschenden Elemente, aber die jüngeren Zellen sind noch ziemlich reichlich. Die Erythroblasteninseln sind viel zahlreicher als die Granulocyteninseln; aus großen Lymphocyten bestehende Inseln sind nicht zu finden. Megakaryocyten sind reichlich vertreten.

Zwischen den Inseln liegen isolierte Zellen der verschiedenen Typen, hauptsächlich Granulocyten; aber hier und dort findet man auch Erythroblasten, kleine Lymphocyten und Polyblasten. Die lebhaft einsetzenden regenerativen Vorgänge äußern sich in dem reichlichen Auftreten von mitotischen Figuren.

Versuch 46: Tötung 8 Tage nach der fünften Injektion.

Auf diesem Schnitt ist die Proliferation noch weiter vorgeschritten. In dem ödematösen Reticulum liegen zehn bis zwölf große, blaugefärbte, mehr oder weniger zirkuläre, aus Parenchymzellen (Taf. I Fig. 7) bestehende

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Inseln. Sie erreichen alle eine beträchtliche Größe und enthalten mehrere hundert Zellen, doch sind die Einzelherde durch weite Flächen voneinander getrennt, in denen die Aplasie deutlich zum Ausdruck kommt. Ihre Anordnung ist keine gleichförmige; sie liegen zentral, intermediär oder peripher. Sie zeigen alle eine Struktur, welche für die größeren Regenerationsherde charakteristisch zu sein scheint; sie bilden keine feste, kompakte Masse, sondern ein ziemlich dichtes Netzwerk. Es beruht dies darauf, daß die Zellen die Neigung haben, der Kapillarwand entlang und nicht durch das interkapilläre Reticulum hindurchzuwachsen; indem sie so den Linien des Kapillarstrombetts folgen, bilden sie keine festen Zellmassen, sondern vielmehr Gruppen von anastomosierenden Zellsträngen. Man erkennt diese Art des Wachstums deutlich am Rande der einzelnen Herde, wo von der Hauptzellmasse aus in mehr oder weniger speichenartiger Anordnung, Bänder von Zellsträngen sich vorschieben (Taf. I Fig. 17). Mit einer Ausnahme sind die größeren beschriebenen Herde alle Reinkulturen von Granulocyten. Nahe an der Peripherie dieser Herde findet man eine oder mehrere typische kleine Erythroblasteninseln; nur in einem der großen Herde treten Gruppen der beiden Zelltypen gleichzeitig auf. Hier scheint es, als wenn der proliferierende Granulocytenherd durch einen vorher von kleinen Erythroblasteninseln eingenommenen Bezirk hindurchgewachsen wäre.

Neben diesen großen Granulocyteninseln findet man einen anderen Wachstumstypus, bei welchem diese Zellen in anastomosierenden Strängen liegen, die von der Peripherie nach dem Zentrum hineinstrahlen (Taf. I Fig. 8). Wo diese Entwicklung schon vorgeschritten ist, reichen die Stränge von der Kapsel bis zum Zentralsinus; wo sie weniger weit vorgeschritten ist, sind sie hauptsächlich auf die Peripherie beschränkt. Ursache dieses ausgedehnten Netzwerks ist wieder die Neigung der Zellen dem Lauf der Kapillaren entlang zu wachsen. Bei diesen Zellgruppen sieht man die Beziehung zum Blutgefäß besonders gut; fast jeder Strang befindet sich in Berührung mit dem Endothel eines dünnwandigen Gefäßes. Die einzelnen Stränge bestehen entweder aus Erythroblasten oder aus Granulocyten; erstere herrschen vor.

Zwischen den großen Granulocytenherden verstreut, liegen in geringer Zahl typische kleine Inseln, wie sie in den früheren Schnitten zu sehen waren. Sie bestehen aus Granulocyten oder Erythroblasten.

Was die Zusammensetzung der großen Granulocytenherde betrifft, so enthalten sie alle Zellen der Granulocytenreihe, vom Myeloblasten bis zum voll entwickelten polynukleären Leukocyten. Hier findet man große Gruppen amphophiler Myelocyten, dort große Gruppen amphophiler Polynukleärer; wieder in anderen Teilen des Schnitts findet man beide Typen innig miteinander vermischt. Myeloblasten sind relativ viel weniger zahlreich als in den früheren Schnitten; sie bilden keine unabhängigen Inseln, sondern finden sich unter den höher differenzierten Zellen versprengt. Eosinophile Myelocyten und Polynukleäre sind verhältnismäßig selten. Alle Typen sind ebenso im peripheren Netzwerk wie in den kleinen Inseln zu finden. Unter den Erythroblastenzellen herrschen die Normoblasten bei weitem vor. Viele zeigen kleine, pyknotische Kerne. In einem nicht geringen Teil der Zellen haben diese Kerne eine partielle Auflösung erlitten, wobei eine blasse Struktur mit tief blauem, halbmond- oder ringförmigem Rand zurückgeblieben ist. Frei im Gewebe liegend, oft in großen Gruppen oder in den Leibern von Phagocyten enthalten, findet man Strukturen, die sich ebenso färben wie diese partiell gelösten Kerne.

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Megakaryocyten: Riesenzellen sind in diesem Präparat außerordentlich reichlich vorhanden. Eine kleine Zahl derselben ist in den großen regenerativen Granulocytengruppen eingeschlossen; viel reichlicher finden sie sich jedoch am Rand solcher Gruppen oder zwischen zwei beieinander liegenden Gruppen. Eine Regelmäßigkeit ist hinsichtlich ihrer Verteilung nicht zu erkennen. Hier treten sie einzeln auf, dort in großen Nestern von 10 bis 15 Zellen. Solch ein Nest enthält Zellen verschiedener Typen; solche mit dunkeln und fast nackten Kernen und solche mit blassem Kern und reichlichem Protoplasma. Phagocytose runder und polynukleärer Zellen erfolgt häufig, und die von einigen Zellen ausgehenden, langen, unregelmäßigen Ausläufer verraten amöboide Tätigkeit. Sowohl uni- wie multipolare Kernfiguren sind zu finden, und viele Megakaryocyten lassen dadurch, daß ihr Protoplasma zerfallen und ihr Kern partiell aufgelöst ist, eine mehr oder weniger weit vorgeschrittene Degeneration erkennen.

Pigment ist in ziemlicher Menge vorhanden, und viele Phagocyten enthalten sowohl Pigment wie auch die vorstehend beschriebenen Kernreste. Kleine Lymphocyten und Polyblasten findet man hier und dort verstreut. Im ganzen Schnitt finden sich zahlreiche Mitosen.

	Weiße Blutzellen	Rote Blutzellen
Normal	3000	4 800 000
Nach Beendigung der Injektionen	720	3 900 000
Am Tage des Todes	720	4 000 000

Zusammenfassung: Die regenerativen Veränderungen sind bei diesem Versuch viel weiter vorgeschritten.

Aplastische Zonen sind zwar noch vorhanden, aber die Parenchymelemente sind viel reichlicher als vorher. Sie treten auf:

1. Als große zirkuläre Inseln, die einige hundert Zellen enthalten,
2. als anastomosierende Netzwerke von Strängen, die von der Peripherie aus zum Zentralsinus hineinstrahlen,
3. als kleine, versprengte Inseln,
4. als isolierte Zellen.

Die großen Inseln sind nahezu Reinkulturen von Granulocyten. Sie bilden weniger ein festes Zellnetz, als ein grobes Netzwerk, eine Erscheinung, die darauf beruht, daß die Zellen weniger Neigung haben, durch das interkapilläre Reticulum hindurch, als vielmehr entlang der Grenze des Kapillarstrombetts zu wachsen. Die höher differenzierten Zelltypen, Myelocyten, Metamyelocyten und Polynukleären herrschen bei weitem vor.

Das anastomosierende Netzwerk von Zellsträngen an der Peripherie enthält Granulocyten und Erythroblasten in annähernd der gleichen Zahl, wobei wieder die ältesten Zelltypen reichlicher sind. Die Bildung

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des Netzwerks beruht wieder auf der Neigung der proliferierenden Zellen, der Grenze des Kapillarbetts zu folgen. Anscheinend schreitet hier das Wachstum von der Peripherie zum Zentrum hin, fort; wo nämlich die Entwicklung nicht weit fortgeschritten ist, liegen die Zellstränge hauptsächlich in der peripheren Zone, wobei das eine Ende oft unmittelbar an die Kapsel des Marks angrenzt.

Die kleinen versprengten Inseln und die isolierten, zwischen ihnen befindlichen Zellen gleichen in jeder Hinsicht den bei den früheren Schnitten beschriebenen. Mitosen erfolgen in großer Zahl. Megakaryocyten sind sehr reichlich und finden sich in allen Entwicklungsstadien. Viele zeigen phagocytische Tätigkeit und einige haben pseudopodenartige Fortsätze — ein Anzeichen amöboider Tätigkeit. In einigen der Zellen findet man unipolare und pluripolare Mitosen.

Die interessantesten Erscheinungen sind die Größe der Granulocyteninseln, die ausgeprägte Neigung beider Zelltypen, den Wänden der Kapillaren entlang zu wachsen und das zunehmende Vorherrschen der älteren Zelltypen.

Versuch 47: Tötung 7 Tage nach der achten Injektion.

In einigen der Segmente beginnt das Mark ein hyperplastisches Aussehen zu zeigen, indem dicke, anastomosierende Zellstränge das Reticulum von der Peripherie bis zum Zentrum hin ausfüllen. In anderen Segmenten gleicht die Anordnung mehr der bei Versuch 46 beschriebenen. Hier und dort sind kleine Zonen, die noch völlig oder fast völlig aplastisch sind. Sie finden sich stets in Beziehung zu einer mittelgroßen Arterie, die zentral oder exzentrisch in der aplastischen Fläche liegen kann. In der Umgebung jeder einzelnen größeren Arterie im Schnitt findet man solch eine Zone von relativer oder absoluter Aplasie. Granulocyten und Erythroblasten sind gleich zahlreich. Unter ihnen sind die jüngeren Zelltypen relativ spärlich.

	Weiße Blutzellen	Rote Blutzellen
Normal	6500	5 700 000
Nach Beendigung der Injektionen	720	5 223 000
Am Tage des Todes	720	3 504 000

Versuch 44: Tötung 11 Tage nach der vierten Injektion (s. Fig. 3 p. 11).

Der Schnitt zeigt starke Hyperplasie; Parenchymzellen füllen fast das ganze Mark aus. Hier und da sind in geringer Zahl Fettzellen versprengt. In der Umgebung von zwei ziemlich kleinen Arterien findet sich ein Gebiet mit relativ stark ausgeprägter Aplasie und hier zeigt das Reticulum noch Anzeichen von toxischem Ödem. Alle Zelltypen sind proliferiert und reichlich vorhanden.

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	Weiße Blutzellen	Rote Blutzellen
Normal	10 000	5 000 000
Nach Beendigung der Injektionen	280	5 130 000
Am Tage des Todes	3400	4 144 000

Versuch 41: Tötung 15 Tage nach der neunten Injektion.

Das Bild ist vollständig das einer extremen Hyperplasie; im ganzen Schnitt finden sich dicke Massen dicht zusammengepackter Parenchymzellen. Fettzellen sind nicht reichlich. Die Hauptbestandteile des hyperplastischen Marks sind polynukleäre Leukocyten und Normoblasten.

	Weiße Blutzellen	Rote Blutzellen
Normal	6000	5 600 000
Nach Beendigung der Injektionen	520	5 616 000
Am Tage des Todes	5920	5 672 000

Versuch 56: Tötung 3 Tage nach der fünften Injektion.

Parenchymelemente sind noch sehr reichlich vorhanden. Im allgemeinen findet man keine scharf umschriebenen Zellgruppen und Zellinseln; auch keine deutlichen Unterschiede zwischen Inseln zweier Typen wie bei den vorher beschriebenen Präparaten. Statt dessen treten die Zellen in diffusen Gruppen auf, die miteinander durch Stränge und Streifen in Verbindung stehen oder unregelmäßig überall verstreut sind.

Herde kernhaltiger roter Blutzellen finden sich in relativ kleiner Zahl; wo sie aber zugegen sind, gleichen sie den bei anderen Versuchen gefundenen Erythroblastenherden. Sie bestehen hauptsächlich aus Normoblasten mit gut erhaltenen Kernen und aus einer verhältnismäßig geringen Zahl der jüngeren Zelltypen. Die Erythroblasten sind überschattet von den zahlreich anwesenden Granulocyten. Unten diesen herrschen typische polynukleäre Leukocyten bei weitem vor. Es folgen die Metamyelocyten, wogegen Myelocyten weniger reichlich und große Lymphocyten relativ sehr spärlich sind. Alle diese Zellen treten auf entweder in schlecht definierten Gruppen oder zu zweien und dreien in allen Teilen des Schnittes verstreut. Eosinophile sind spärlich. Megakaryocyten sind in geringer Zahl zugegen; dagegen finden sich viele kleine Lymphocyten und Polyblasten.

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	Weiße Blutzellen	Rote Blutzellen
Normal	10 000	4 800 000
Nach Beendigung der Injektionen	840	—
Am Tage des Todes	3000	4 010 000

Zusammenfassung: Der Versuch muß mit den Versuchen 50 und 53 verglichen werden, da diese im allgemeinen eine entsprechende Erholungsdauer darstellen. Das Ergebnis unterscheidet sich von dem der beiden genannten Versuche.

1. in dem reichlichen Auftreten von Parenchymelementen,
2. in dem starken Vorherrschen der höher differenzierten Zellen,
3. im Fehlen der Inselbildung.

Die rasche Entwicklung einer so großen Zahl von Zellen der älteren Typen, (Polynukleäre, Metamyelocyten und Normoblasten), entspricht den in normalem Mark des Erwachsenen sich abspielenden Vorgängen, wenn solch ein Mark durch einen Reiz zu gesteigerter Aktivität veranlaßt wird. Augenscheinlich läßt sich der Befund durch die Annahme erklären, daß in diesem Fall die Aplasie niemals einen vorgeschrittenen Grad erreicht hat; und daß zur Zeit der Beendigung der Injektionen noch eine große Anzahl von Parenchymzellen bestand, die zu weiterem Wachstum und mitotischer Tätigkeit fähig waren. Gleichzeitig mit der raschen Zunahme der Parenchymelemente ist in den 3 auf die Beendigung der Injektionen folgenden Tagen eine Vermehrung der weißen Zellen des zirkulierenden Bluts von 840 auf 3000 im cmm erfolgt.

Allgemeine Zusammenfassung:

Benzol zerstört die spezifischen Knochenmarkszellen. Eine Serie von Injektionen hat den äußersten Grad von Aplasie des Marks zur Folge.

Wie viele andere Gifte, wirkt es aber nicht nur als zerstörendes Agens, sondern auch als Reiz; in der absteigenden Reihe sind Reizung und Zerstörung überall miteinander verknüpft. Anfangs herrscht erstere vor, und die Parenchymelemente nehmen außerordentlich rasch an Zahl zu (Taf. I Fig. 4). Hauptsächlich vermehren sich, wie bei der Hyperplasie des normalen Marks des Erwachsenen die höher differenzierten Zelltypen, wogegen die jüngeren Formen Myeloblasten und Megaloblasten, weniger betroffen sind.

Schon am Ende des zweiten Tages ist in diesem hyperplastischen Mark eine deutlich ausgeprägte Zellzerstörung wahrzunehmen. Mit zu-

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nehmender Zahl der Dosen wird von dieser Zeit an eine stets zunehmende Zahl von Zellen geschädigt. Die geschädigten Elemente verschwinden allmählich und lassen die Zellstränge zerrissen und ungeordnet zurück. Sie sind voneinander getrennt durch weite Flächen ödematösen Reticulums und dilatierter Kapillaren. Schließlich bleiben nur isolierte Zellen oder kleine Gruppen von zweien oder dreien zurück. Nach der neunten Injektion ist das Mark fast völlig aplastisch (Taf. I Fig. 3). Alle Zelltypen, Erythroblasten, Granulocyten und Megakaryocyten werden durch das Gift geschädigt. Mit Ausnahme der polynukleären Elemente scheinen alle in annähernd gleichem Maße betroffen zu sein. Bei den polynukleären Amphophilen ist die Zahl der degenerierenden Zellen immer relativ geringer als bei den anderen Zelltypen. Nichtsdestoweniger nimmt ihre Zahl nach der zweiten Injektion im Mark außerordentlich rasch ab — eine Erscheinung, die vermutlich darauf zurückzuführen ist, daß sie in großer Menge in das zirkulierende Blut ausgeschwemmt werden. Aus diesem Grunde sind die amphophilen Myelocyten, obgleich empfindlicher als die Polynukleären, doch die in den früheren Stadien vorherrschenden Zellen.

In all diesen Beispielen der absteigenden Reihe findet man im Reticulum verstreut beträchtliche Mengen kleiner Lymphocyten und Polyblasten (s. Zusammenfassung bei Versuch 39).

Ob diese Zellen normale Bestandteile des Marks oder ob sie aus Blutgefäßen eingewandert sind, ließ sich nicht feststellen. Welches aber auch ihr Ursprung sein mag, jedenfalls zeigen sie eine größere Widerstandsfähigkeit gegen das Gift als die Parenchymelemente; sie bestehen und nehmen anscheinend an Zahl noch zu, wenn schon alle anderen Zellen zerstört sind.

Nach Beendigung der Injektionen beginnen regenerative Prozesse, welche eine Rückkehr des Marks zur Norm bewirken. Die Geschwindigkeit, mit welcher sich die regenerativen Vorgänge äußern, ist in den einzelnen Fällen eine verschiedene. Wo die Vergiftung weniger schwer gewesen ist (Versuch 56), werden die geschädigten Zellen rasch durch proliferierende Gruppen normaler Zellen ersetzt. Wo als Ergebnis der Vergiftung eine ausgesprochene Aplasie eingetreten ist, sind für den Eintritt einer wirksamen Regeneration 3—6 Tage erforderlich. Die Zeitdauer hängt ab von 2 Faktoren: erstens von der Zahl der noch bestehenden Parenchymelemente, zweitens von der Menge des bei Beendigung der Injektionen noch zirkulierenden Benzols.

In den frühen Stadien der Regeneration erscheinen die Zellen im aplastischen Reticulum in Form von umschriebenen Inseln und Gruppen (Fig. 5, 6, 18, 19). Diese sind mehr oder weniger weit voneinander getrennt, extravaskulär und berühren oft die Wände der Kapillaren. Sie bestehen aus Erythroblasten, Granulocyten oder großen Lymphocyten; am zahlreichsten sind Erythroblasteninseln.

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Zwischen diesen Inseln liegen sowohl isolierte Zellen der verschiedenen Typen, wie kleine Lymphocyten und Polyblasten. Mit fortschreitender Regeneration nehmen die Inseln an Zahl und Größe zu (Taf. I Fig. 7). Sie liegen nahe aneinander, und sich berührende Gruppen haben das Bestreben, miteinander zu verschmelzen. Der Zelltypus, ob Erythroblast oder Granulocyt, bleibt, nachdem die Proliferation begonnen hat, konstant. Neben den größeren und kleineren Inseln findet sich ein anderer Wachstumstypus, bei welchem von der Peripherie ausgehende Zellstränge in nach innen einstrahlenden Bändern gegen den Zentralsinus hinwachsen (Taf. I Fig. 8).

Im ganzen Verlauf des regenerativen Prozesses sind Megakaryocyten zugegen. Sie können überall im Gewebe isoliert auftreten oder deutliche Inseln bilden. In diesen Inseln findet man nicht nur voll entwickelte Zellen mit kompliziertem Kernbild und reichlichem Protoplasma, sondern auch die jüngeren Formen, bei denen der Kern rund oder oval und das Protoplasma relativ spärlich ist. Zwischen diesem Typus und der ausgewachsenen Zelle sieht man alle Arten von Übergängen. Sowohl multipolare wie unipolare Mitosen treten auf. Einige der Zellen sind phagocytär, und einige lassen amöboide Tätigkeit erkennen.

Wenngleich in den Frühstadien die höher differenzierten Zellen (Myelocyten, Polynukleäre und Normoblasten) vorherrschen, so sind die jüngeren Formen (große Lymphocyten, Myeloblasten, Megaloblasten) relativ sehr zahlreich. Mit fortschreitender Regeneration bleiben die jüngeren Zellen zwar bestehen, bilden aber einen stetig abnehmenden Prozentsatz des Zellgehalts des Marks. In allen Stadien findet man zwischen den Inseln stets isolierte Zellen von all den verschiedenen Typen.

Die weiteren Ereignisse bestehen einfach in einer Fortsetzung des beschriebenen Prozesses, so daß das Mark nach Aufhören der Injektionen in einem Zeitraum von 10—18 Tagen nicht nur seinen ursprünglichen Zellgehalt wieder erreicht, sondern auch noch hyperplastisch wird und sich von der Peripherie bis zum Zentrum als mit Zellen beladen erweist.

Ein Vergleich der absteigenden mit den früheren Beispielen der aufsteigenden Reihe zeigt deutlich, daß in einem Mark, das einen vorgeschrittenen Grad von Aplasie erreicht hat, Regeneration erfolgen kann.

Die frühen regenerativen Veränderungen sind, wie gesagt, durch Bildung kleiner, umschriebener Zellnester und Zellinseln charakterisiert. Die Bildung solcher Inseln in einem aplastischen Mark kann nur auf eine Weise erklärt werden, nämlich durch die Annahme, daß jede Insel sich aus einer einzelnen Mutterzelle entwickelt. Welches ist nun der Ursprung und die Natur dieser Mutterzelle?

Wegen der Verschiedenheiten in der Wirkung einer bestimmten Anzahl von Giftdosen, kann man in keinem Falle annehmen, daß alle Markzellen vor Beginn der Regeneration verschwunden sind. Wahrscheinlich bleiben stets einige Parenchymzellen erhalten, die noch zu

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mitotischer Tätigkeit fähig sind. Das Bestehen dieser Zellen kann die Bildung der Erythroblasten- und Granulocyteninseln, oder wenigstens einiger dieser Inseln, wohl erklären. In den früheren Stadien der Regeneration sind aber auch große Lymphocyten reichlich anwesend, sowohl in Form von Inseln, wie im Gewebe versprengt. Im normalen Mark des Erwachsenen findet man verhältnismäßig wenige dieser Zellen, und während der Verabreichung des Giftes degenerieren sie zusammen mit den anderen Zelltypen. Man muß also annehmen, daß sie zur Zeit der Beendigung der Injektionen sehr spärlich vorhanden sind. Welches ist dann aber der Ursprung dieser Zellen? Berücksichtigt man die vorausgegangenen zerstörenden Veränderungen, so erscheint die Zahl der großen Lymphocyten in den frühen Stadien der Regeneration zu reichlich, und sie finden sich zu weit versprengt, als daß man annehmen könnte, sie seien von überlebenden großen Lymphocyten abzuleiten.

Von anderen in Betracht kommenden Ursprungsquellen können die fixen Gewebszellen (Bindegewebe und Endothel) ausgeschlossen werden. Sie zeigen zu keiner Zeit irgendwelche Anzeichen von Tätigkeit; man findet unter ihnen niemals mitotische Figuren, und ebensowenig sieht man Übergangsformen. Was von den fixen Gewebszellen gilt, gilt in gleicher Weise von den Klasmatocyten, wie bei Schnitt 39 (Zusammenfassung) gezeigt wurde. Es verbleiben also kleine Lymphocyten und Polyblasten, Zellen, die in beträchtlicher Zahl in allen Beispielen der absteigenden Reihe auftreten. Ihr Ursprung ließ sich nicht mit Sicherheit ermitteln. Sei es aber, daß sie überlebende Parenchymelemente darstellen, sei es, daß sie als Derivate der kleinen Lymphocyten des zirkulierenden Bluts anzusprechen sind, jedenfalls sind es die widerstandsfähigsten aller Zelltypen, und sie sind noch anwesend, nachdem alle anderen Zellen verschwunden sind. Ihre Verteilung als versprengte, isolierte Exemplare stimmt überein mit der Verteilung der Zellen im regenerierenden Mark, und es scheint sehr möglich oder sogar sehr wahrscheinlich, daß sie die Hauptrolle bei den regenerativen Veränderungen spielen. Eine Umwandlung von kleinen Lymphocyten in große würde das reichliche Auftreten der letzteren völlig erklären, und das Zustandekommen einer solchen Umwandlung wird allgemein als ein physiologischer Prozeß betrachtet, wie er z. B. in den Lymphdrüsen sich abspielt. Eine weitere Differenzierung der so gebildeten großen Lymphocyten in Zellen der Erythroblasten- und Granulocytenreihe ist für die Vertreter der monophyletischen Theorie ein Vorgang, dessen Erklärung keine Schwierigkeiten darbietet. Maximow (30) hat gezeigt, daß der Prozeß sich so abspielen kann bei der Bildung des Knochenmarks in der Niere von Kaninchen.

Das Studium der Megakaryocyten führt zu derselben Auffassung. Diese Zellen verdanken ihre Entstehung nicht der Teilung präexistierender Zellen desselben Typus vgl. Saxer (45), sondern einer Weiterentwicklung

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großer Lymphocyten. Sie werden leicht zerstört, erscheinen aber in dem regenerierenden Mark in beträchtlicher Zahl. Ihre Anwesenheit läßt wieder auf ein reichliches Auftreten der großen Lymphocyten im regenerierenden Mark schließen und zur Erklärung dieser Verhältnisse müssen andere Dinge herangezogen werden, als das Bestehen der wenigen großen Lymphocyten, die etwa noch am Leben geblieben sind. Die nach unserer Ansicht wahrscheinlichste Erklärung ist oben gegeben worden: eine Umwandlung der kleinen Lymphocyten in große und eine weitere Differenzierung der letzteren.

V. Lymphdrüsen.

Allgemeines. Die Lymphdrüsen, welche in den untersuchten Fällen studiert wurden, entstammten der Radix mesenterii M, der großen und kleinen Kurvatur des Magens S, der Inguinalgegend J, der oberflächlichen Fascie des Oberschenkels L, und gelegentlich auch anderen Regionen. Da die Veränderungen von Tag zu Tag ziemlich langsam fortschreiten, so sollen nur diejenigen Schnitte betrachtet werden, deren Beschreibung für die Gewinnung einer allgemeinen Vorstellung von dem Prozeß in seinen typischen Stadien erforderlich ist. In vielen Fällen zeigen Lymphdrüsen, die verschiedenen Gegenden ein und desselben Tieres entnommen sind, beträchtliche Unterschiede. Im allgemeinen sollen aber diese Unterschiede bei der Beschreibung unberücksichtigt bleiben, und es soll in der Regel nur eine typische Drüse als Beispiel eines jeden Versuchs beschrieben werden.

A. Degenerative Veränderungen.

Versuch 29: Tötung 1 Tag nach der ersten Injektion.

Die Drüse sieht etwas hyperplastisch aus; die Zellen sind sehr reichlich und dicht zusammengepackt. Die einzelnen Follikel sind groß und bestehen hauptsächlich aus einem großen, runden oder ovalen Keimzentrum, das von relativ wenigen Strängen kleiner Lymphocyten umgeben ist. Kernfiguren sind nicht häufiger als in der normalen Drüse. In der ganzen Drüse finden sich große und kleine Lymphocyten, deren Kerne verschiedene Grade von Pyknose, Karyolyse und Karyorrhexis zeigen. Als Ergebnis dieses degenerativen Prozesses findet man Chromatinfragmente überall im Gewebe verstreut; sie liegen teils frei, teils sind sie in Phagocyten enthalten (Taf. III Fig. 20).

Versuch 30: Tötung 24 Stunden nach der dritten Injektion.

Die Keimzentren treten in allen Follikeln deutlich als große, zentrale, sich schwächer färbende Zonen hervor, die von relativ wenigen Strängen kleiner Lymphocyten umgeben sind. Überall in der Follikelgegend finden sich degenerierende Lymphocyten und Kernfragmente, doch sind sie viel weniger zahlreich als im vorhergehenden Schnitt. Die meisten Fragmente liegen frei; verhältnismäßig wenige sind von Phagocyten aufgenommen. Das

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lymphatische Gewebe der Follikelgegend beginnt eine Reduktion seiner Masse zu zeigen. Die Lymphocyten in den Follikeln sind weniger kompakt angeordnet, als es normalerweise der Fall ist und die ganze Follikelzone ist etwas geschrumpft. Die Markstränge sind wenig verändert.

Versuch 33: Tötung 24 Stunden nach der siebenten Injektion.

M. Kernfragmente sind etwas zahlreicher als in dem eben beschriebenen Beispiel. Sie treten sowohl in den Follikeln wie in den Marksträngen auf.

Der Lymphocytengehalt der Drüse ist deutlich reduziert. Die Lymphocyten sind in den Follikeln sehr locker angeordnet und die retikulären Zellen und das retikuläre Netzwerk treten deutlich hervor. Die ganze Follikelzone ist deutlich abgeflacht und geschrumpft. Sie hat sich eine Strecke weit von der Kapsel retrahiert, so daß der periphere Sinus weit offen steht (Taf. II Fig. 14). An der Stelle der Vereinigung der Follikel und Stränge sind die Marksinus dilatiert und die Follikelzone wird durch sie zum Teil gespalten. In den Follikeln selbst sind ähnliche dilatierte Lymphräume vorhanden.

Nicht alle Follikel sind in demselben Maße geschädigt. Hier und dort findet man einen, der sich vor seinen Nachbarn durch größeren Reichtum an Lymphocyten auszeichnet. Die Markstränge sind weniger betroffen.

Versuch 35: Tötung 24 Stunden nach der achten Injektion.

Die Follikel sind nicht alle in demselben Maße betroffen. Einige zeigen noch ein ziemlich normales Aussehen; andere lassen eine erhebliche Abnahme ihres Lymphocytengehalts erkennen — eine Abnahme, die an der Peripherie oder auch im Zentrum ausgesprochener sein kann (Taf. II Fig. 13—14). In denjenigen Follikeln, die am schwersten geschädigt sind, sind die Lymphocyten fast völlig verschwunden und die Lage des Follikels kann nur an der Anordnung des retikulären Netzwerks erkannt werden. Die Markstränge zeigen ähnliche Veränderungen.

Versuch 39: Das Tier starb kurz nach der neunten Injektion.

Die Kapsel ist verdickt und ödematös; ihre Fasern sind auseinander geschoben und mit Serum infiltriert, und ihre Bindegewebszellen sind vom Typus der Fibroblasten. Im peripheren Teil der Kapsel sind sie relativ spärlich, aber in der unmittelbaren Umgebung der Drüsensubstanz bilden sie ein dicht gepacktes Zelllager mit wenig Intercellularsubstanz. Zwischen den Fibroblasten liegen, verstreut und in Gruppen, rote Blutzellen, polynukleäre Leukocyten und Polyblasten. Von der Kapsel aus nach innen strahlend sieht man Bänder ähnlichen Gewebes, welche die Follikel voneinander trennen und in die Follikel selbst eindringen.

Der einzelne Follikel zeigt ein ganz anormales Aussehen. Er ist partiell oder vollständig umgeben von einer Kapsel jungen Bindegewebes, welche in seine Peripherie hineingreift und Sprossen in seine Substanz hinein sendet. Die Zellen im Follikel sind locker angeordnet und bestehen aus kleinen Gruppen kleiner Lymphocyten, einigen wenigen großen Lymphocyten und einer wechselnden Anzahl von Polyblasten und Plasmazellen. Die Markstränge zeigen ähnliche Veränderungen. Sie sind ödematös und geschwollen und greifen auf die Marksinus über. Sie bestehen hauptsächlich aus jung aussehendem, cellulärem Bindegewebe, in dem nur gelegentlich Fibrillenbänder auftreten. Auch hier liegen in den Gewebsmaschen in ziemlicher Menge Polyblasten und Plasmazellen.

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B. Regenerative Veränderungen.

Versuch 56: Tötung 3 Tage nach der fünften Injektion.

Die ganze Follikelzone ist in ihrem Umfang verringert und die Lymphocyten liegen weit voneinander getrennt im retikulären Netzwerk. Hier und dort findet man dichtere, kompaktere Gruppen Lymphocyten, die eine mehr zentrale Stellung im Follikel einnehmen. Die umgebenden Gebiete sind arm an Zellen und das retikuläre Netzwerk tritt deutlich hervor.

Die Bildung dieser dichteren Zellgruppen stellt vielleicht den Beginn der regenerativen Veränderungen dar.

Versuch 51: Tötung 5 Tage nach der achten Injektion.

M. Die Kapsel der Drüse ist ödematös und das Bindegewebe vermehrt. Einige der Lymphfollikel zeigen eine Reduktion ihrer Größe und Erweiterung der peripheren Sinus; in anderen ist die Menge lymphatischen Gewebes nicht wesentlich unter die Norm herabgesunken. Die Keimzentren treten nicht hervor und mitotische Figuren sind nicht häufig.

L. Die regenerativen Veränderungen in dieser, demselben Tier entnommenen Drüse sind viel weniger vorgeschritten als in der eben beschriebenen. Sowohl Aplasie wie Fibrose sind deutlicher ausgesprochen. Kapsel, Trabekel, Follikel und Markstränge sind von der Fibrose betroffen; an einigen Stellen ist das Bindegewebe in die medullären Lymphräume hineingewachsen und hat sie ganz oder teilweise zur Obliteration gebracht.

Versuch 47: Tötung 7 Tage nach der achten Injektion.

M. Die Kapsel ist geschwollen ödematös und enthält neu gebildetes Granulationsgewebe. Das lymphatische Gewebe ist ziemlich reichlich, aber die Follikel haben ihre normale Größe noch nicht völlig wieder erlangt. Von einer geringen Zunahme an Bindegewebszellen abgesehen zeigen sie aber ein annähernd normales Aussehen. Keimzentren treten ziemlich deutlich hervor, doch scheinen die Mitosen in ihnen nicht wesentlich vermehrt zu sein. In den Marksträngen findet man beträchtliche Mengen neu geformten jungen Bindegewebes; am reichlichsten findet sich dasselbe aber zwischen den Strängen, wo es ein ziemlich dichtes, maschiges Netzwerk bildet, welches viele der Marksinus ausfüllt.

Versuch 41: Tötung 15 Tage nach der neunten Injektion.

M. Der größere Teil des Schnittes zeigt normales Drüsengewebe (Taf. II Fig. 12). Doch sind einige der Follikel klein, unregelmäßig und undeutlich begrenzt, und von der Kapsel retrahiert, wobei der Sinus weit offen steht. Auch sind die Zellen hier weniger kompakt. An diesen Stellen ist die Wiederherstellung keine vollständige.

Pigment: In vielen Lymphdrüsen findet man fein- und grobkörniges Pigment von gelber bis gelblichbrauner Farbe. Seine Menge und Verteilung variiert in den verschiedenen Drüsen bei ein und demselben Versuch außerordentlich. Es kann liegen in dem Protoplasma großer Zellen, die in den Follikeln und Marksträngen in Gruppen auftreten, in freien Phagocyten der peripheren oder medullären Lymphräume, in den Leibern und Fortsätzen der retikulären Zellen, welche in den

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Sinus ein loses anastomosierendes Netzwerk bilden; und endlich in den Endothelzellen. Erythrocyten finden sich, teils frei, teils in den Leibern von Phagocyten, in wechselnder Zahl in den Lymphräumen der Drüsen. Da normale Drüsen ähnliche Unterschiede in der Menge und Verteilung von Pigment und Erythrocyten aufweisen, so kann den genannten Schwankungen wenig Bedeutung beigelegt werden, weshalb von einer näheren Beschreibung abgesehen werden soll.

C. Allgemeine Zusammenfassung.

Injektion von Benzol bewirkt sowohl in den Follikeln wie in den Marksträngen der Lymphdrüsen eine Zerstörung der Lymphocyten. Innerhalb von 24 Stunden nach der ersten Injektion tritt in den Zellen Karyolyse und Karyorrhexis auf, und man findet Chromatinfragmente überall in den Geweben verstreut. Nach weiteren Injektionen sind diese Zeichen von Degeneration viel weniger ausgeprägt. Ein Teil der Zelltrümmer wird von großen Phagocytenzellen aufgenommen und verdaut.

Nach der dritten Injektion beginnt der Zellgehalt der Follikel eine deutliche Reduktion aufzuweisen. In dem Maße, wie die Zellen zerstört werden, zieht sich das Retikulum, in welchem sie lagen, zusammen; es erfolgt eine Abflachung der ganzen follikulären Zone. Die peripheren und medullären Sinus erleiden eine kompensatorische Dilatation. Mit Fortschreiten des Prozesses werden die Lymphocyten allmählich weniger zahlreich und schließlich bleibt nur das Retikulum zurück, um die frühere Lage der Follikel anzuzeigen. Die Markstränge leiden weniger als die Follikularzone (Taf. II Fig. 13, 14).

Etwa am 8. oder 9. Tage zeigen die Lymphdrüsen ausgesprochene Aplasie. Der Prozeß vollzieht sich aber nicht in gleichmäßiger Weise und noch in den vorgeschrittensten Stadien der Degeneration existieren Follikel, in denen eine beträchtliche Menge von Lymphocyten erhalten geblieben ist.

Nach Beendigung der Injektionen erfolgt eine allmähliche Regeneration der Lymphocyten. In den aplastischen Drüsen erscheinen sie in 3—4 Tagen als kompakte Zellgruppen, die in der Follikulärzone mehr oder weniger zentral gelagert sind. In dem Maße wie sie an Zahl zunehmen, füllen sie das retikuläre Netzwerk aus, und die Follikelzone gewinnt wieder ihre normale Größe. In 10—15 Tagen ist die Regeneration vollständig (Taf. II Fig. 12). In vielen Drüsen ist jedoch eine völlige Rückkehr zur Norm unmöglich. Infolge der durch das Gift bewirkten Reizung bildet sich in Kapsel, Strängen, Follikeln und Marksinus Granulationsgewebe, welches eine ausgesprochene Fibrose zur Folge hat.

Im großen und ganzen sind die Veränderungen in den Lymphdrüsen weniger ausgesprochen als im Knochenmark. Noch in den am weitesten vorgeschrittenen Degenerationsstadien finden sich Follikel, in denen eine beträchtliche Zahl von Lymphocyten erhalten geblieben ist, und wenn

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auch einige der Follikel sich erst in 10—15 Tagen nach Beendigung der Injektionen vollständig regenerieren, so gibt es doch auch solche, die sich viel rascher erholen. Ein Vergleich der beiden Reihen zeigt, daß die Follikel, nachdem sie fast völlig aplastisch gemacht worden sind, sich doch wieder erholen können.

VI. Appendix (Lymphfollikel).

A. Normale Verhältnisse.

Vor Beschreibung der in der Appendix auftretenden Veränderungen sollen kurz die normalen Verhältnisse besprochen werden (Taf. II Fig. 9). CZERMACK (8) sagt bei Beschreibung der Appendix des normalen Kaninchen: „Vom Peritoneum abgesehen, sind alle Teile durch die Follikel verzerrt. Die Muskelschicht ist schwach entwickelt, die Muscularis mucosae fehlt fast vollständig und das Epithel ist an vielen Stellen deformiert."

Der größte Teil der Wand der Appendix wird von der Submucosa gebildet. Die in Taf. II Fig. 9 a, b, c, d, bezeichneten Bezirke stellen Teile des lymphodenoiden Gewebes der Submucosa dar, die der Bequemlichkeit halber getrennt beschrieben werden sollen.

a) Dies sind große ovale Strukturen, die durch eine dünne Schicht von Bindegewebe scharf abgegrenzt sind. Die Follikel bestehen aus ziemlich locker angeordneten kleinen und großen Lymphocyten, die in einem zarten Reticulum [1]) liegen. Außer diesen Lymphocyten sieht man die runden oder ovalen, bläschenförmigen Kerne retikulärer Zellen, gelegentlich polynukleäre Amphophile und Pigmentzellen. Die Pigmentzellen findet man in Form von runden oder unregelmäßigen Zonen, von blaßgelber bis dunkelgelber Farbe (Taf. II Fig. 9 E); sie grenzen sich von dem umgebenden lymphatischen Gewebe scharf ab. Im allgemeinen liegen sie in der Peripherie der Follikel. Die einzelnen Zellen haben einen Durchmesser von 10—14 μ und sind von polygonaler Gestalt. Ihre Kerne sind ziemlich klein, rund oder oval und bläschenförmig. Die gelbe Farbe der Zelle beruht auf der Ansammlung dicht gepackter Massen blaßgelber bis kupferfarbiger Körner in ihrem Cytoplasma. In diesen Zellen ist weder die Farbe noch die Größe der Pigmentkörner eine gleichmäßige. Zellen von ähnlichem Aussehen findet man häufig in den Follikeln und Marksträngen der Lymphdrüsen und in der Milz.

Außer den beschriebenen zellulären Bestandteilen sind stets kleine Mengen von Kernfragmenten zugegen, die in Größe und Färbbarkeit variieren (tingible Körper).

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[1]) Nach CZERMACK (8) sind in diesem Bezirk hauptsächlich die mit den Zellen des Keimzentrums identischen großen Lymphocyten angesammelt, so daß er wirklich das Keimzentrum des Follikels darstellt.

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b) Der Bezirk gleicht den Follikeln eines gewöhnlichen Lymphknotens, indem er fast ausschließlich aus kleinen, zu einer dichten Masse zusammengepackten Lymphocyten besteht.

c) Hier ist die Anordnung der Zellen wieder eine lockere und gleicht mehr der bei a). Die kleinen Lymphocyten sind weniger kompakt und die großen reichlicher.

d) Dieser Bezirk enthält lediglich eine kompakte Masse kleiner Lymphocyten.

Mucosa. Unter normalen Verhältnissen findet man viele Lymphocyten im Bindegewebe der Mukosa verstreut wie auch zwischen den Epithelzellen, welche die Zotten bedecken und die LIEBERKÜHN'schen Drüsen auskleiden.

B. Degenerative Veränderungen.

Versuch 29: Tötung 24 Stunden nach der ersten Injektion.

Unter schwacher Vergrößerung erscheint der Schnitt normal. Bei starker Vergrößerung sieht man Veränderungen, die den bei den Lymphdrüsen des entsprechenden Vergiftungsstadiums beschriebenen außerordentlich gleichen. Sie bestehen in einer Degeneration der Lymphocyten; Pyknose, Karyolyse, Karyorrhexis- und in der Gegenwart von Chromatinmassen, welche als Folge dieser Degeneration auftreten. Auch findet man einige Phagocyten mit Kernfragmenten. Das lymphatische Gewebe wird nicht gleichmäßig betroffen; nur Bezirk a) leidet in ausgedehnterem Maße. Wie gesagt findet man unter normalen Bedingungen viele kleine Lymphocyten, die im Bindegewebe der Mucosa liegen oder zwischen die Epithelzellen der Zotten und LIEBERKÜHN'schen Drüsen auswandern. In dem vorliegenden Schnitt ist die Zahl dieser Lymphocyten wesentlich reduziert.

Versuch 30: Tötung 24 Stunden nach der dritten Injektion.

Es zeigt sich eine deutliche Reduktion der Zahl der Lymphocyten, welche das Bindegewebe der Mucosa infiltrieren und zwischen das Epithel der Zotten und LIEBERKÜHN'schen Drüsen auswandern. Die Follikel zeigen auch einen reduzierten Lymphocytengehalt; nicht nur sind die Lymphocyten weniger kompakt angeordnet als in der Norm, sondern es ist auch die gesamte Dicke der Submucosa vermindert. In dem Maße wie die Lymphocyten verschwinden, erfolgt eine allgemeine Zusammenziehung des Reticulums, in welchem sie sich befanden, so daß die Submucosa sich verdünnt und atrophisch wird. Eine derartige Veränderung ist analog dem, was in den Follikeln der Lymphdrüsen gefunden wurde.

Wie man aus den Ergebnissen des vorhergehenden Versuchs schon vermuten konnte, erfolgt der größte Verlust an Zellen in dem mit a) bezeichneten Teil des Follikels. Die Lymphocyten in diesem Bezirk sind zu einem großen Teil verschwunden. Sie teilweise ersetzend, findet man viele große, mononukleäre Zellen mit rundem oder ovalem, sich schwach färbenden Kern und ziemlich reichlichem, rosa gefärbtem, körnig aussehendem Protoplasma.

Zellen mit gelbem, körnigem Pigment, wie sie als im normalen Appendix auftretend beschrieben wurden, sind in sehr kleiner Zahl zugegen.

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Versuch 31: Tötung 24 Stunden nach der vierten Injektion.

Der Schnitt zeigt eine auffällige Abweichung von der Norm und ein weiteres Fortschreiten der Veränderungen, wie sie im vorhergehenden Fall sichtbar waren. Das Bindegewebe der Mucosa ist außerordentlich arm an Lymphocyten und nur gelegentlich sieht man eine Zelle von diesem Typus durch das Epithel wandern.

Die Submucosa zeigt eine deutliche Abnahme ihrer Dicke (Taf. II Fig. 10). Die Follikel, welche ihre Hauptmasse bilden, haben überall einen großen Teil ihres Lymphocytengehaltes verloren. Die Veränderung ist am wenigsten in d) und bei weitem am deutlichsten in a) ausgesprochen, wo man Lymphocyten nur als isolierte Exemplare findet. In diesem Teil des Follikels ist das Reticulum ausgefüllt mit großen, mononukleären Zellen ähnlich denen, wie sie in ähnlicher Lagerung im vorigen Schnitt beschrieben wurden (Taf. II Fig. 10a). Hier und dort findet man morphologisch sehr ähnliche Zellen, welche in ihrem Cytoplasma eine wechselnde Menge gelber Pigmentkörner enthalten und welche den Pigmentzellen des normalen Appendix gleichen. Zwischen den pigmentierten und nicht pigmentierten Zellen bestehen alle Arten von Übergängen. Degenerative Veränderungen in den Kernen sind häufig.

Versuch 39: Das Tier starb kurz nach der neunten Injektion.

Der Schnitt stellt einen extremen Grad des in den beiden vorhergehenden Schnitten beobachteten Prozesses dar (Taf. II Fig. 11). In der Mucosa treten nur ausnahmsweise Lymphocyten auf. Die Submucosa hat mit einer normalen kaum noch irgendwelche Ähnlichkeit. Sie ist dünn und atrophisch und ihre Lymphocyten sind fast gänzlich zerstört oder ausgeschwemmt. Nur in dem mit d bezeichneten Bezirk treten noch kleine Gruppen auf. Die mononukleären Zellen und die Pigmentzellen, welche vorher nur im Basalteil des Follikels anwesend waren, sind jetzt in den ganzen Follikel eingedrungen.

C. Regenerative Veränderungen.

Versuch 59: Tötung 2 Tage nach der vierten Injektion.

Die Mucosa zeigt einige Zellen, die im Bindegewebe versprengt sind und durch das Epithel hindurchwandern. Die Submucosa besitzt vielleicht ein Drittel ihrer normalen Dicke; Hand in Hand mit dieser Atrophie geht eine bedeutende Reduktion der Zahl der Lymphocyten. Wie gewöhnlich, ist diese Reduktion am stärksten ausgeprägt an der Basis des Follikelbezirks. Mononukleäre Zellen, wie sie vorhin beschrieben wurden, sind hier in beträchtlicher Zahl zugegen; viele von ihnen sind degeneriert und vakuolisiert. Berücksichtigt man, daß dies Tier nur 4 Dosen Benzol erhielt, so würde man auf Grund des Zustands der Appendix zu dem Schluß kommen, daß nur eine sehr geringe Regeneration stattgehabt hat.

Versuch 56: Tötung 3 Tage nach der fünften Injektion.

Die Lymphocyten zeigen überall die Neigung an Zahl zuzunehmen; die Zellen liegen dicht beieinander und das lymphatische Gewebe scheint etwas gleichförmiger. An der Basis des Follikels ist der Zellgehalt noch ein sehr niedriger.

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Versuch 51: Tötung 5 Tage nach der achten Injektion.

Die Regeneration macht rasche Fortschritte. Überall im Follikel. ausgenommen an der Basis, sind die Lymphocyten dicht zusammengepackt und das ganze Aussehen ist ein gleichförmiges und regelmäßiges. In diesen Bezirken sind reichlich mitotische Figuren vorhanden. Die Submukosa hat etwa die Hälfte ihrer normalen Dicke wieder erlangt. Im interstitiellen Gewebe der Mukosa sind Lymphocyten nicht reichlich.

Versuch 47: Tötung 7 Tage nach der achten Injektion.

Das Aussehen beginnt sich der Norm zu nähern. Im Bindegewebe der Mukosa und zwischen den Epithelzellen findet man jetzt eine große Anzahl von Lymphocyten. Das lymphatische Gewebe der Submukosa hat ein gleichförmiges, kompaktes Aussehen und sogar an der Basis des Follikels beginnen sich die Lymphocyten in beträchtlicher Zahl anzusammeln. Sie nehmen den zentralen Teil dieses Bezirks ein, wogegen die Peripherie mit pigmentierten und nicht pigmentierten großen mononukleären Zellen ausgefüllt ist. Mitosen sind reichlich vorhanden.

Versuch 44: Tötung 11 Tage nach der vierten Injektion. Normale Appendix.

D. Zusammenfassung.

Das lymphatische Gewebe der normalen Appendix besteht aus großen Follikeln, die fast die ganze Submukosa erfüllen (Taf. II Fig. 9). Die unter der Einwirkung der Vergiftung eintretenden Veränderungen in diesem Gewebe gleichen durchaus den für die Lymphdrüsen beschriebenen. Sie bestehen in zerstörenden Prozessen, welche zu einer vorgeschrittenen Aplasie führen und denen eine vollständige Regeneration folgt. Bei allen Versuchen zeigt der basale Teil der Submukosa a) die größten Veränderungen. Nach der ersten Injektion findet man in der Submukosa viele degenerierende Lymphocyten. Unter der Einwirkung weiter fortgesetzter Injektionen nehmen die Lymphocyten allmählich an Zahl ab, das Reticulum tritt hervor und die ganze Submukosa zieht sich zusammen. In dem Maße wie die Lymphocyten verschwinden, füllt sich das Reticulum teilweise mit pigmentierten und nicht pigmentierten, großen, mononukleären Zellen (Taf. II Fig. 10a). Zwischen den pigmentierten und nicht pigmentierten Zellen findet man alle Arten von Übergängen. Die pigmentierten Zellen sind identisch mit denen des normalen Appendix. Die Tatsache, daß das Reticulum mit diesen Zellen angefüllt ist, bedeutet demnach nicht ein Eindringen fremder Elemente, sondern eine Zunahme an Elementen, die schon normalerweise vorhanden sind. Hand in Hand mit diesen Veränderungen geht ein allmählicher Rückgang der Zahl der in der Mukosa liegenden Lymphocyten. Nach neun Injektionen ist der größte Teil des lymphatischen Gewebes zerstört und es bestehen nur noch einzelne wenige versprengte Gruppen von Lymphocyten. Die Submukosa hat sich auf einen

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kleinen Teil ihrer ursprünglichen Dicke kontrahiert (Taf. II Fig. 11).

Regenerative Veränderungen treten 3—4 Tage nach Beendigung der Injektionen in Erscheinung; sie bestehen in einer Umkehr des zerstörenden Prozesses: in einer allmählichen Ansammlung von Lymphocyten in der Submukosa und im Wiedererscheinen ähnlicher Zellen im Bindegewebe und im Epithel der Mukosa. Gleichzeitig mit ihrer Zunahme in den Follikeln gewinnen die Lymphocyten eine kompaktere Anordnung und ein gleichmäßigeres Aussehen; die Follikel selbst erweitern sich, die Submukosa beginnt sich auszudehnen und die Wand der Appendix erlangt wieder ihre normale Dicke. 10—14 Tage nachdem die Verabreichung von Benzol aufgehört hat, ist der regenerative Prozeß beendet.

VII. Milz.

A. Absteigende Reihe.

Versuch 29: Tötung 24 Stunden nach der ersten Injektion.

Bei schwacher Vergrößerung ist das Aussehen ein normales, abgesehen von einem etwas ungewöhnlichen Hervortreten der Keimzentren der MALPIGHI'schen Körperchen. Bei stärkerer Vergrößerung sieht man innerhalb der Körperchen eine beträchtliche Anzahl von Lymphocyten, deren Kerne geschrumpft und pyknotisch sind; ferner findet man Gruppen von Chromatinfragmenten, die von einem Abbau dieser Zellen herrühren. Auch in der Pulpa sieht man einige pyknotische Kerne.

In dem Milzsinus finden sich viele Lymphocyten und polynukleäre Amphophile von normalem Aussehen, sowie einige große mononukleäre Zellen, die ein feinkörniges gelbes Pigment enthalten.

Die Pulpastränge sind weit, sehen normal aus und enthalten:

1. viele typische kleine Lymphocyten;
2. große Zellen mit reichlichem Protoplasma, die sich schwach anfärben und unscharf abgegrenzt sind. Die Kerne sind von runder, ovaler oder hufeisenförmiger Gestalt; sie sind groß, bläschenförmig und sehr arm an Chromatin. Einige dieser Zellen sind phagocytär; sie haben gelbliche Pigmentkörner oder gelegentlich weiße Blutzellen aufgenommen. Sie sollen als große mononukleäre Pulpazellen bezeichnet werden;
3. Viele rote Blutzellen;
4. polynukleäre Amphophile;
5. polynukleäre Eosinophile in geringer Zahl;
6. retikuläre Zellen.

Die Zahl der degenerierenden Formen ist viel weniger als in den Lymphdrüsen und in der Appendix in dem entsprechenden Vergiftungsstadium.

Versuch 30: Tötung 24 Stunden nach der dritten Injektion.

Es läßt sich eine deutliche Einbuße an lymphatischem Gewebe feststellen. Die Größe der MALPIGHI'schen Körperchen ist stark reduziert; die Lymphocyten sind in beträchtlicher Zahl nur in der Umgebung des zentralen Gefäßes angesammelt. Der Rand dieser Zellgruppe ist unregelmäßig und

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ausgezackt; innerhalb der Gruppe sind kleine zellfreie Räume. Unter den zurückgebliebenen Zellen sind einige pyknotisch, andere sehen normaler aus. Die mehr peripheren Teile der MALPIGHI'schen Körperchen bestehen aus Reticulum mit konzentrisch angeordneten, bläschenförmigen Kernen, unter denen verstreut sich einige kleine Lymphocyten befinden. Kernfragmente sind in geringer Zahl zugegen.

Pulpastränge. Alle wichtigen Elemente, Lymphocyten, große mononukleäre Pulpazellen, polynukleäre Amphophile und rote Blutzellen sind noch anwesend. Doch ist ihre Zahl vermindert und die Stränge sind dünner als normal, so daß die Kerne des Reticulums und Endothels relativ mehr hervortreten. Einige der Polynukleären zeigen Pyknose und Karyorrhexis. Degenerierende Lymphocyten sind, wenngleich in geringerer Zahl, gleichfalls zugegen.

Sinus. Die Sinus sind nicht dilatiert. Ihr Gehalt, sowohl an Lymphocyten wie an Polynukleären ist reduziert; unter beiden Zellarten finden sich Exemplare, die Degeneration erkennen lassen.

Versuch 27: Tötung 24 Stunden nach der sechsten Injektion.

Das Beispiel zeigt ausgeprägtere Veränderungen als die vorher untersuchten. In den MALPIGHI'schen Körperchen ist die Zahl der Lymphocyten stark reduziert; nur wenige einigermaßen kompakte Reihen umgeben und begleiten die Zentralarterie. Anderswo tritt das retikuläre Netzwerk des Körperchens ohne Lymphocyten deutlich hervor. Pyknotische und degenerierende Zellen sind spärlich. In den Maschen des Reticulums sieht man einige Polyblasten und Plasmazellen (Taf. II Fig. 16).

Die Milzpulpa zeigt analoge Veränderungen. Die Pulpastränge sind dünn und zart und bestehen hauptsächlich aus dem kollabierten Reticulum, in welchem rote Blutzellen liegen. Lymphocyten, große mononukleäre Pulpazellen und polynukleäre Amphophile findet man nur hier und dort als isolierte Exemplare.

Die Endothelkerne ragen in die Sinus vor, welch letztere mit Erythrocyten bepackt sind. Auch hier sind Lymphocyten spärlich, aber immer noch viel reichlicher als polynukleäre Amphophile. Von beiden Zellarten findet man degenerierte Exemplare. Mitotische Figuren sind selten.

Versuch 39: Das Tier starb wenige Stunden nach der neunten Injektion.

In diesem Beispiel findet man als Wirkung wiederholter Dosen des Giftes extreme Veränderungen. Schon bei schwacher Vergrößerung sieht man, daß die MALPIGHI'schen Körperchen statt dunkler als das umgebende Gewebe gefärbt zu sein, heller sind. Das Zentralgefäß liegt in dem lockeren, retikulären Netzwerk mit seinen ovalen, bläschenförmigen Kernen. In den Maschen dieses Netzwerkes liegen einige versprengte Lymphocyten.

Pulpastränge. An Stelle von weiten, aus lockerem Reticulum bestehenden und mit roten Blutzellen, Lymphocyten usw. bepackten Strängen findet man eine dünne kollabierte kompakte Wand. In dieser Wand sind die Maschen des Reticulums eng, so daß die Kerne des Endothels und Reticulums zahlreicher zu sein scheinen als sie normalerweise sind. In den Maschen liegen einige isolierte Erythrocyten. Hier und dort sind die roten Blutzellen im Bindegewebe an der Berührungsstelle einer Gruppe von Sinus zahlreicher und zuweilen findet man einige Lymphocyten oder auch eine große mononukleäre Pulpazelle (Taf. II Fig. 17).

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Sinus. Sie sind dilatiert, mit Blut gefüllt und enthalten außerordentlich wenig Lymphocyten. Polynukleäre Leukocyten findet man weder hier noch in den Pulpasträngen.

Der Schnitt zeigt fast völlige Aplasie der Milz. Lymphocyten sind sowohl in den MALPIGHI'schen Körperchen wie in den Pulpasträngen und in den Sinus nur in außerordentlich geringer Zahl anwesend. Polynukleäre Amphophile fehlen völlig.

B. Regenerative Veränderungen.

Versuch 53: Tötung 4 Tage nach der siebenten Injektion.

Die MALPIGHI'schen Körperchen zeigen eine beträchtliche Anzahl von Lymphocyten, die sich in ziemlich gleichförmigen, kompakten Massen in unmittelbarer Umgebung des Zentralgefäßes angesammelt haben; der periphere Teil des Follikels enthält dagegen verhältnismäßig wenige solcher Zellen, und der Gesamtlymphocytengehalt des Follikels ist wesentlich geringer als normal. Die Keimzentren treten nicht hervor und Mitosen sind nicht zahlreich.

Die Pulpastränge sind dünner als normal und die Kerne des Reticulums und des Endothels treten infolge der Abnahme der anderen zelligen Elemente deutlich hervor. Erythrocythen sind in beträchtlicher Zahl zugegen, wogegen Lymphocyten nur hier und dort in kleinen Gruppen zu finden sind. Polynukleäre Amphophile sind spärlich und große mononukleäre Pulpazellen sind nur gelegentlich zu finden.

Die Sinus sind nicht dilatiert. Außer den roten Blutzellen enthalten sie überall kleine Mengen von Lymphocyten und einige polynukleäre Amphophile.

Unter Berücksichtigung der Zahl der Injektionen — sieben — lehrt ein Vergleich mit dem entsprechenden Beispiel in der absteigenden Reihe, daß die Regeneration bereits in vollem Gange ist. Die Zahl der Lymphocyten ist zwar noch reduziert, sie sind aber viel zahlreicher als die polynukleären Elemente, sowohl in den Sinus- wie in den Pulpasträngen.

Versuch 52: Tötung 5 Tage nach der fünften Injektion.

Die MALPIGHI'schen Körperchen kehren sehr rasch zur Norm zurück. Die Menge des lymphatischen Gewebes ist, wenn auch noch erheblich reduziert, doch bereits viel größer als im vorhergehenden Beispiel.[1]) Hier und dort findet man in dem Körperchen kleine Gruppen von Polyblasten und Plasmazellen. Mitotische Figuren sind nicht reichlich.

Die Pulpastränge sind breiter und die retikulären Zellen treten weniger deutlich hervor. Zwischen ihnen liegen viele Erythrocyten und eine ansehnliche Zahl von Lymphocyten, polynukleären und großen mononukleären Pulpazellen. Auch in dem Sinus zeigen die Lymphocyten und Polynukleären eine entschiedene Zunahme gegenüber der Zahl im vorhergehenden Beispiel.

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[1]) Beim Vergleich dieses Schnittes mit dem vorhergehenden muß die Differenz in der Zahl der Injektionen berücksichtigt werden; das mehr dem Normalen sich nähernde Aussehen dieser Milz beruht wahrscheinlich ebenso sehr auf der geringeren Zahl der Injektionen, wie darauf, daß ein Tag mehr zur Erholung zur Verfügung stand.

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Versuch 44: Tötung 11 Tage nach der vierten Injektion.

Die Regeneration ist weiter vorgeschritten als bei den vorhergehenden Beispielen. Die MALPIGHI'schen Körperchen haben ein fast normales Aussehen. Die Pulpastränge sind breit und ihr Zellgehalt ist nur wenig reduziert (Taf. II Fig. 15). In dem Sinus nimmt die Zahl der weißen Blutzellen zu; die Lymphocyten sind noch reichlicher als die Polynukleären.

In geringer Zahl im Schnitt verstreut, sowohl einzeln wie in Gruppen von dreien und vieren, findet man Zellen, die in keinem der bisher betrachteten Fälle in der Milz beobachtet wurden. Das vorherrschende Element in diesen Gruppen ist eine große, runde oder annäherend ovale Zelle von 12—15 μ Durchmesser. Der Kern ist verhältnismäßig groß und ist rund, oval oder hufeisenförmig; er besitzt ein zartes Chromatinreticulum. Charakteristisch ist das Protoplasma, welches eine gleichmäßig verteilte, dichte Masse feiner, lilafarbiger Körner (H und E) enthält. Das Aussehen gleicht völlig dem des amphophilen Myelocyten des Knochenmarks (Taf. III Fig. 23h). Zusammen mit diesen Zellen oder auch getrennt von ihnen finden sich kleine Gruppen ähnlich gebauter Zellen, deren Leiber aber mit groben, lebhaft roten Körnern gefüllt sind — eosinophile Myelocyten — (Taf. III Fig. 23 g). Die beschriebenen Zellen findet man entweder frei in dem Sinus oder im Reticulum des Pulpastrangs. Sie fehlen in den MALPIGHI'schen Körperchen, wenn man auch hier und dort einen isolierten Myelocyt in einer der äußersten Reihen von Lymphocyten findet, die das Körperchen umgrenzen. Die Sinus enthalten überall in der Milz viele kernhaltige rote Blutzellen und zugleich einige typische Knochenmarksmegakaryocyten.

Die kernhaltigen roten Blutzellen sind hauptsächlich solche mit relativ kleinen, pyknotischen Kernen und reichlichem Protoplasma, also eher ältere als jüngere Typen von Erythroblasten. Sie liegen innerhalb der Sinus und sind in dem ganzen Organ mehr oder weniger gleichmäßig verteilt. An vielen Stellen bieten sie ein bemerkenswertes Bild dar; — sie erscheinen nicht mehr als abgerundete Scheiben, sondern zeigen Sanduhr- oder Hantelform (Taf. III Fig. 21, 22 c). Das eine Ende der Hantel liegt innerhalb des Pulpastranges; es besteht lediglich aus Chromatin und variiert in seiner Größe zwischen einem bloßen Chromatinflecken und einer Masse von der Ausdehnung eines ganzen Normoblastenkerns (Taf. III Fig. 23 C). Von ihm führt ein zarter Chromatinstreif durch die Sinuswand [1]) hindurch in den Sinus hinein. Er dringt hier in den Leib des Normoblasten ein und endet mit einer knollenartigen Schwellung, die aus dem Rest des Normoblastenkernes besteht. Die Menge des im Zellleib enthaltenen Kernmaterials steht in umgekehrten Verhältnis zu der in der Pulpa enthaltenen Menge. Die Länge des Chromatinstreifens wechselt; meist ist er kurz, zuweilen aber erreicht er eine Länge von 6 – 7 μ.

[1]) Nach WEIDENREICH (54) ist die Wand des Sinus vollständig geschlossen und besteht a) an der Innenseite aus langen, schlanken Endothelzellen, die parallel der Längsachse des Sinus verlaufen. Sie stehen nicht in direkter Verbindung miteinander, sondern sind durch relativ breite Zwischenräume getrennt, b) aus einer dünnen, strukturlosen Membran, auf welcher diese Zellen ruhen. Diese Membran zeigt Stomata, die auf dem Durchtritt farbloser Körperchen beruhen, c) aus dicken cylindrischen Fasern, die zum Reticulum der Markstränge gehören und die in Ebenen verlaufen, welche senkrecht zu den Endothelzellen gerichtet sind.

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Der Zellleib kann seine normale Gestalt behalten oder in die Form eines Dreiecks ausgezogen sein (Taf. III Fig. 21 c). In diesem Falle geht der Chromatinstreifen von der Spitze des Dreiecks aus. In keiner dieser Zellen begleitet das Protoplasma den Chromatinstreifen durch die Sinuswand. Alles Protoplasma liegt vielmehr innerhalb des Sinus, und sowohl der Streifen wie die Chromatinmasse in dem Pulpastrang sind extrazellulär. Alle Kerne, welche diese Veränderungen aufweisen, nehmen eine gleichmäßige tiefblaue Färbung an und zeigen keinerlei Struktur. Gelegentlich findet man in dem Sinus eine Zelle, die den hantelförmigen Normoblasten gleicht und auch einige nackte Kerne finden sich hier (Taf. III Fig. 21 f). Wie bereits erwähnt, kommen in dem Sinus kernhaltige rote Blutzellen vom gewöhnlichen Typus vor; in den Pulpasträngen findet man aber so gut wie keine solche Zellen. In den Strängen liegen eine beträchtliche Anzahl nackter Normoblastenkerne.

Zusammenfassung: Die Regeneration ist fast vollständig. Außer ihren normalen Zellen enthält die Milz amphophile und eosinophile Myelocyten, Megakaryocyten und kernhaltige rote Zellen. Unter den letzteren finden sich viele, die eine Hantelform zeigen, wobei das Protoplasma mit einem Teil des Kerns das eine Ende der Hantel bildet und innerhalb des Sinus gelegen ist. Der übrige Teil des Kerns bildet (nackt) das andere Ende der Hantel und liegt innerhalb des Pulpastrangs. Diese beiden Enden stehen miteinander in Verbindung durch einen Chromatinstreifen, welcher die durch die Sinuswand hindurchgehende Zusammenschnürung bildet.

Versuch 41: Tötung 15 Tage nach der neunten Injektion.

Die regenerativen Veränderungen in der Milz sind so gut wie vollständig (Taf. II Fig. 15).

Hier und dort in der Pulpa verstreut findet man einige Zellen, die in jeder Hinsicht den beim vorigen Schnitt als amphophile Myelocyten beschriebenen gleichen. Die Gegenwart dieser Zellen im Verein mit einer kleinen Anzahl von Normoblasten und Megakaryocyten läßt an eine beginnende myeloide Metaplasie denken. Eosinophile Myelocyten sind abwesend.

Eine kleine Zahl von Normoblasten zeigt Hantelform und dieselbe Beziehung zur Pulpa, Sinus und Sinuswand, wie sie viele der im vorhergehenden Abschnitt beschriebenen Erythroblasten zeigten.

Versuch 42: Tötung 19 Tage nach der fünften Injektion.

Der allgemeine Bau und das Aussehen der Milz sind normal. Der Regenerationsprozeß ist vollständig. Doch zeigt sich eine etwas weniger als in Schnitt 44 ausgeprägte myeloide Metaplasie.

Pigment: In allen Schnitten der Milz tritt in wechselnder Menge gelbes bis gelbbraunes Pigment auf. Es findet sich hauptsächlich in den Pulpasträngen entweder frei oder in Phagocyten. In geringerem Maße findet man Pigmentzellen in dem Sinus und peripheren Zonen der Malpighi'schen Körperchen. Die späteren Beispiele zeigen einen höheren Pigmentgehalt als die früheren. Aber zu keiner Zeit besteht gegenüber der Norm eine sehr bedeutende Vermehrung.

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Milz. Allgemeine Zusammenfassung.

Die durch das Gift in der Milz hervorgerufenen Veränderungen sind ähnlich denen wie sie in den Lymphdrüsen und in der Appendix gefunden wurden. Sie bestehen im wesentlichen aus einem allmählich eintretenden Verlust an Parenchymelementen, der mit fast völliger Aplasie des Organs endet und dem vollständige Regeneration folgt. Weiterhin sind noch zwei Punkte von Interesse:

a) eine in den späteren regenerativen Stadien auftretende myeloide Metaplasie, und

b) eine damit einhergehende besondere Beziehung der kernhaltigen roten Blutzellen zu der Sinuswand.

Nach den beiden ersten Injektionen treten die Keimzentren ungewöhnlich hervor. Kernfragmente und degenerierende Kerne sind viel weniger zahlreich als in den Lymphdrüsen in den entsprechenden Vergiftungsstadien. Diese Abweichung ist, wenn man bedenkt, daß das Endergebnis der Vergiftung in den beiden Organen ein ähnliches ist, schwer zu erklären. Die degenerierenden Zellen finden sich hauptsächlich in den MALPIGHI'schen Körperchen, in geringerem Maße auch in den Pulpasträngen. Nur wenige dieser degenerierten Zellen werden von Phagocyten aufgenommen.

In den MALPIGHI'schen Körperchen verschwinden die Lymphocyten zuerst von der Peripherie des Follikels und lassen um das Zentralgefäß herum eine kompakte Gruppe von Zellen zurück. Mit fortschreitender Vergiftung vermindert sich allmählich die Zahl der die Zentralarterie umgebenden Zellen (Taf. II Fig. 16) und nach neun Injektionen sind fast alle Lymphocyten zerstört, so daß die Lage des Follikels nur noch an der Anordnung des Reticulums und des Zentralgefäßes zu erkennen ist.

Hand in Hand mit den Veränderungen in den MALPIGHI'schen Körperchen geht eine Zerstörung der Parenchymzellen in den Marksträngen. Lymphocyten, große mononukleäre Pulpazellen und Polynukleäre verschwinden allmählich. Das Reticulum zieht sich zusammen und die Stränge schrumpfen, so daß die Zellen des Reticulums und Endothels mehr hervortreten (Taf. II Fig. 17). In den späteren Stadien werden sogar die Erythrocyten ausgeschwemmt. Die Aplasie der Stränge wird eine vollständige am Ende des 9. Tages, also gleichzeitig mit der Aplasie der MALPIGHI'schen Körperchen. Dies ist von Wichtigkeit, weil die Veränderungen in den Strängen nicht so früh sichtbar werden wie die in den Follikeln wie das schon HEINEKE hervorgehoben hat (S. 683). Der Unterschied beruht aber wahrscheinlich nicht wie HEINEKE behauptet, auf einer Verschiedenheit in der Wirkung des Gifts auf die beiden Teile der Milz, sondern auf der Tatsache, daß geringe Veränderungen leichter in den Follikeln als in den Strängen zu erkennen sind.

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Nach Aufhören der Injektionen nehmen die Parenchymelemente der Markstränge und MALPIGHI'schen Körperchen allmählich zu. Nach 10—15 Tagen bietet die Milz wieder ein so gut wie normales Bild dar. Ein Vergleich der Versuche 41 und 39 zeigt, welche regenerative Fähigkeiten die Milz besitzt. Versuch 39 zeigt die wenige Stunden nach der neunten Injektion herrschende fast völlige Aplasie; Versuch 41 zeigt eine normale Milz 15 Tage nach der neunten Injektion (Taf. II Fig. 15).

In den letzten Beispielen der aufsteigenden Reihe, nämlich denen, bei welchen die Tiere 11, 15 und 19 Tage nach der letzten Darreichung des Gifts getötet wurden, zeigte sich eine myeloide Metaplasie der Milz — d. h. die Entwicklung typischer Knochenmarkselemente — amphophilen und eosinophilen Myelocyten, Normoblasten und Megakaryocyten (Taf. III Fig. 23 g, h, i). Die Myelocyten treten sowohl isoliert auf wie auch in kleinen Gruppen, in welchen sie mit den normalen Elementen der Pulpa und der Sinus vermischt sind. Die kernhaltigen roten Blutzellen und die Megakaryocyten finden sich in den dem Sinus.

Eine solche myeloide Metaplasie ist in der neueren Literatur häufig beschrieben worden (s. Einl.). Nach MEYER und HEINEKE (34) und nach DOMARUS (11) ist das myeloide Gewebe in der Milz autochthonen Ursprungs; es stellt die Rückkehr des Organs zu seiner embryonalen hämatopoetischen Funktion dar und ist kompensatorischer Natur. STERNBERG (49) neigt der Ansicht zu, daß die Gegenwart von myeloiden Zellen in der Milz in vielen Fällen mit ihrer Funktion als Blutfilter zusammenhängt und nicht mit ihrer Funktion als hämatopoetisches Organ. In den oben beschriebenen Fällen dürfte aber ein autochthoner Ursprung der Zellen das Wahrscheinlichere sein, da die Zahl der im Blut zirkulierenden unreifen Blutzellen (Myelocyten, Normoblasten) eine sehr geringe ist.

Für eine kompensatorische Natur des Prozesses ist die Gesamtzahl der in der Milz gefundenen myeloiden Zellen nicht groß und wenn man die außerordentliche Hyperplasie des Knochenmarks bei den entsprechenden Versuchen in Betracht zieht, so erscheint es unwahrscheinlich, daß die Milz viel zu der Regeneration des Blutes sollte beigetragen haben.[1]) Wie MEYER und HEINEKE (34) gezeigt haben, ist nur die rote Milzpulpa — Stränge und Sinus — an dem Prozeß beteiligt. Die MALPIGHIschen Körperchen sind nicht betroffen, wenn man auch in den oben beschriebenen Beispielen gelegentlich einen isolierten Myelocyten in der äußersten Peripherie des Follikels findet.

[1]) Am Tage des Todes ausgeführte Blutzählungen ergaben in diesen Fällen:

	rote	weiße Blutzellen
Versuch 44:	4 144 000	3 400
„ 41:	5 672 000	5 920
„ 42:	4 256 000	4 000

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Unter den kernhaltigen roten Blutzellen sind viele, die eine hantelförmige Gestalt besitzen und die teilweise im Sinus, teilweise im Pulpastrang liegen. Der im Pulpastrang liegende Teil besteht aus einem nackten Kern und ist extrazellulär. Von ihm aus dringt ein Streifen nackten Chromatins durch die Sinuswand hindurch in den Sinus und hier in den Zellleib ein. Hier vereinigt er sich mit dem Rest des Kerns, welcher von dem gesamten Zellprotoplasma umgeben ist. Innerhalb der Pulpastränge liegen nackte Kerne, aber keine kernhaltigen roten Blutzellen. (Taf. III Fig. 21, 22, 23).

Für eine Erklärung dieses Bildes kommen drei Möglichkeiten in Betracht. Es kann darstellen:

I. Diapedese der kernhaltigen roten Blutzellen.
II. Eine aktive Wanderung der Zelle.
III. Eine Ausstoßung oder Auswanderung des Kernes aus dem Zellleib.

I. Diapedese.

Das Bild gleicht in auffälliger Weise dem einer Kapillare des Frosches nach Abbindung der größeren Vene, in welche diese Kapillare mündet. In dem Gefäße dicht hinter der Ligatur füllen sich die Kapillaren und Venen mit kernhaltigen roten Blutzellen.[1]) Darauf erfolgt Diapese (THOMA (52) und man findet viele kernhaltige rote Blutzellen in den Wänden der Kapillaren stecken, wo sie sanduhrförmig zusammengeschnürt sind (Taf. III Fig. 24).[2]) Der Zellteil, welcher zuerst austritt, besteht hauptsächlich oder ganz aus Protoplasma; er kann einen kleinen Teil des Kernes enthalten. Der ganze Kern oder der noch übrige Teil des Kernes — wie es im einzelnen Fall sich gerade trifft — bleibt im Lumen der Kapillare zurück. Es ist dies aber nicht ein nackter Kern, sondern ein Kern, der von einer deutlichen Schicht Protoplasma umgeben ist. Es teilt sich demnach der Zellleib in zwei Teile; dabei kann der Kern ungeteilt bleiben oder mitgeteilt werden. Kein Teil des Kernes ist also extrazellulär. Kernhaltige rote Zellen finden sich frei im Gewebe in der Umgebung der dilatierten Kapillaren.

Vergleicht man nun die beiden Erscheinungen miteinander, so zeigt sich beim Frosch folgendes:

1. Infolge einer ausgesprochenen Blutstauung in den Kapillaren erfolgt Diapedese.

[1]) Die Gelegenheit zur Untersuchung und Zeichnung dieses Vorgangs beim Frosch verdankt der Autor der Liebenswürdigkeit von Herrn Prof. MARCHAND, welchem er dafür auch hier seinen Dank auszusprechen wünscht.

[2]) Die roten Blutzellen des Frosches sind größer als die des Kaninchens, von ovaler Form und sämtlich kernhaltig.

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2. Während des Prozesses ist der Zellleib stets in zwei Teile geteilt; der Kern kann geteilt sein, oder ungeteilt bleiben.

3. Kein Teil des Kernes liegt extrazellulär.

4. Kernhaltige rote Zellen finden sich frei in den Geweben.

Im Falle der oben beschriebenen Kaninchenmilz andererseits erfolgt 1. keine Blutstauung, die eine Diapedese erklären könnte. 2. Der Kern ist stets in zwei Teile geteilt, der Zellleib nie. 3. Ein Teil des Kernes liegt stets extrazellulär. 4. In der Pulpa findet man so gut wie keine kernhaltigen roten Zellen. Trotz der scheinbaren Ähnlichkeit der beiden Bilder erscheint demnach mit Rücksicht auf Verschiedenheiten in den Bedingungen ihres Auftretens und mit Rücksicht auf Verschiedenheiten in den Einzelheiten der Vorgänge der Schluß gerechtfertigt, daß die Grunderscheinungen in den beiden Fällen verschieden sind. [1])

II. Aktive Wanderung kernhaltiger roter Blutzellen.

In der Milz finden sich oft Lymphocyten, welche durch die Sinuswände hindurchwandern (Taf. III Fig. 23 k). Wenn kernhaltige rote Zellen, wie die Lymphocyten, aktiv wandern, so erhebt sich die Frage, in welcher Richtung die Bewegung erfolgt. Nimmt man an, daß sie von der Pulpa nach dem Sinus hin gerichtet ist, so müßte das gesamte Protoplasma zuerst hindurchgehen, denn man sieht Beispiele, in denen das im Sinus liegende Protoplasma nur einen kleinsten Flecken von Chromatin enthält, wobei dies mit der Hauptmasse des nackt in dem Pulpastrang liegenden Kernes durch einen Chromatinfaden verknüpft ist (Taf. III Fig. 23 c). Dieser Prozeß würde ein fast vollständiges Herauspressen des Kernes aus dem Zellprotoplasma während des Durchganges der Zelle durch die Sinuswand bedeuten. Die Vollendung des Wanderaktes könnte nur dadurch erfolgen, daß dieser Kern durch die Wand des Sinus hindurchtritt und dann in den Zellleib zurückkehrt.

Wenn die Wanderung in der Richtung vom Sinus zur Pulpa hin erfolgt, so müßte man mit Rücksicht auf Bilder desselben Typus (wo fast der ganze Kern nackt im Pulpastrang liegt und nur noch ein Chromatinfleck im Zellleib vorhanden ist), annehmen, daß zuerst fast der ganze Kern den Zellleib verläßt und durch die Sinuswand in die Pulpa wandert; daß er alsdann mittels einer zarten Verbindungsbrücke aus Chromatin den Zellleib nachzieht, und schließlich in die Zelle, aus der er austrat, zurückkehrt.

Es ist klar, daß diese beiden Möglichkeiten erhebliche Schwierigkeiten darbieten; daß sie tatsächlich als Unmöglichkeiten zu bezeichnen sind. Zudem liegen, wie schon erwähnt, fast alle kernhaltigen roten

[1]) Obgleich eine Diapedese von Erythrocyten allgemein angenommen wird, so konnte WEIDENREICH (54) doch keine Bilder erhalten, die in der Milz auf eine solche schließen ließen; Figuren, die eine Wanderung farbloser Körperchen zeigten, waren dagegen reichlich vorhanden.

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Zellen innerhalb des Sinus und so gut wie gar keine finden sich in den Pulpasträngen; eine Tatsache, die energisch gegen eine Wanderung, gleichgültig in welcher Richtung, spricht. Schließlich existieren auch keinerlei Anzeichen dafür, daß die älteren Formen kernhaltiger roter Zellen amöboide Eigenschaften hätten. Lobenhoffer (28) nimmt diese Möglichkeit für die jüngeren Formen an, bringt aber keinen Beweis dafür. Thayer (57), in einer neu erschienenen Arbeit, teilt eine amöboide Bewegung in Zellen des Megaloblastentypus mit. Diese aktiven Formveränderungen konnte er in dem frisch entnommenen Blut unter dem Mikroskop verfolgen.

Die in den Schnitten als sanduhrförmig beschriebenen Zellen sind solche mit homogenen, sich diffus färbenden Kernen und reichlichem Protoplasma, d. h. die älteren und nicht die jüngeren Typen. All diese Punkte sprechen dagegen, die Erscheinung als eine aktive Wanderung kernhaltiger roter Blutzellen zu betrachten.

III. Ausstoßung oder aktive Wanderung des Kernes aus dem Zellleib.[1])

Für einen solchen Vorgang sprechen eben die Punkte, welche eine Diapedese oder aktive Wanderung der ganzen Zelle als ausgeschlossen erscheinen lassen.

1. Die Tatsache, daß das gesamte Protoplasma stets, unabhängig von seinen Beziehungen zum Kern, in dem Sinus verbleibt.

2. Daß der in der Pulpa liegende Teil des Kernes stets nackter Kern ist; ein Kern, der bereits aus dem Zellleib ausgetreten ist. Wie gesagt kann fast der ganze Kern im Pulpastrang liegen, und nur ein Chromatinfleck im Protoplasma zurückbleiben. Bilder von diesem Typus geben den Eindruck, daß der Kern gerade im Begriff ist, sich ganz von dem Normoblasten zurückzuziehen und ihn als einen Erythrocyten zurückzulassen.

3. Das außerordentlich spärliche Auftreten von kernhaltigen roten Blutzellen in der Pulpa.

4. Das zahlreiche Auftreten nackter Normoblastenkerne in der Pulpa (Taf. III Fig. 21, 23 f).

Diese Tatsachen führen mit Notwendigkeit zur Verwerfung der beiden erst betrachteten Erklärungen und zur Annahme der dritten. Die Bilder stellen eine Auswanderung des Kernes aus dem Leib der roten Blutzelle dar, und stützen demnach die Ausstoßungstheorie im Gegensatz zu der der intracellulären Degeneration des Kernes. Damit soll aber die Möglichkeit eines Auftretens von intracellulärer Degeneration in kern-

[1]) Die Literatur über diese Frage ist in der Einleitung kurz besprochen worden.

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haltigen roten Blutzellen an anderen Stellen des Körpers nicht in Abrede gestellt werden.

Es ist nicht anzunehmen, daß diese Auswanderung des Kernes aus dem Zellleib nur in Beziehung zu der Wand des Milzsinus erfolgt. Hier und dort sieht man sowohl in dem Sinus selbst wie auch an vielen Stellen des Knochenmarks Bilder, die anscheinend einen ähnlichen Prozeß erkennen lassen. Aber an keinem anderen Ort lassen sich die Beziehungen zwischen Kern und Zellleib so genau und sicher bestimmen und nirgendwo anders sprechen sie so deutlich für die Umwandlung des Erythroblasten in den Erythrocyten durch eine aktive Auswanderung des Kernes.

VIII. Andere Organe.

Das Studium der anderen Organe des Körpers ergibt keine besonders wichtigen Resultate. Leber und Niere zeigen wechselnde Grade von fettiger Entartung. Außerdem findet man in einigen der Fälle Blutungen in der Wand des Magens und Darms und in der Lunge.

Schlußzusammenfassung:

Faßt man die Ergebnisse des experimentellen Studiums der Benzolvergiftung kurz zusammen, so sind die wichtigsten Punkte folgende:

1. Benzol ist ein mächtiges Leukotoxin. Es zerstört die weißen Zellen des zirkulierenden Blutes und die Parenchymzellen der hämatopoetischen Organe.

2. Myeloides Gewebe wird schwerer geschädigt als lymphadenoides; dementsprechend werden die polynukleären Leukocyten des zirkulierenden Blutes stärker betroffen als die Lymphocyten. Das erythroblastische Gewebe des Knochenmarks wird zerstört, wogegen die zirkulierenden Erythrocyten relativ wenig geschädigt werden.

3. Durch wiederholte Benzolinjektionen kann das myeloide und das lymphadenoide Gewebe fast völlig aplastisch gemacht werden.

4. Werden die Injektionen beendet, nachdem die hämatopoetischen Organe fast ganz aplastisch geworden sind, so beginnen nach 3—4 Tagen regenerative Veränderungen. Innerhalb von 10 Tagen bis 3 Wochen regenerieren sich diese Organe vollständig. Mit zunehmender Regeneration nähert sich das Blutbild wieder dem normalen.

5. Der regenerative Prozeß im Knochenmark beginnt mit der Bildung kleiner umschriebener Zellgruppen und Zellinseln, die aus großen Lymphocyten, Granulocyten oder Erythroblasten bestehen. Hat die celluläre Differenzierung einmal begonnen, so bleibt in jeder einzelnen solcher Insel der Zelltypus, ob Erythroblast oder Granulocyt, konstant. Die Inseln nehmen an Größe und Zahl zu, verschmelzen miteinander und führen zu einer vollständigen Ausfüllung des Reticulums mit Parenchymelementen. In den frühesten Stadien sind die jüngeren Zelltypen (Myelo-

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blasten und Megaloblasten) verhältnismäßig reichlich. In den späteren Stadien sind diese Zellen zwar auch noch zugegen, aber im Vergleich mit höher differenzierten Zellen sind sie spärlich.

6. Die polynukleären Amphophilen im Mark sind widerstandsfähiger als große Lymphocyten, Myelocyten, Erythroblasten und Riesenzellen. Noch widerstandsfähiger als die polynukleären Amphophilen sind typische kleine Lymphocyten und Polyblasten. Diese beiden Zelltypen findet man noch, nachdem alle anderen Knochenmarkszellen verschwunden sind. Ihr Ursprung, ob sie sich von den Lymphocyten des zirkulierenden Bluts ableiten oder normale Bestandteile des Marks darstellen, konnte nicht mit Sicherheit festgestellt werden. Welches aber auch ihr Ursprung sein mag, jedenfalls spielen diese kleinen Lymphocyten offenbar eine wichtige Rolle bei den regenerativen Prozessen. Wahrscheinlich besteht diese Rolle in einer Umwandlung kleiner Lymphocyten in große und in einer weiteren Differenzierung dieser letzteren in Granulocyten, Erythroblasten und Megakaryocyten.

7. Während der späteren Stadien der Regeneration findet man in der Milz myeloide Zellen aller Typen. Sie sind relativ gering an Zahl und das Knochenmark in den entsprechenden Fällen ist hyperplastisch. Die myeloide Metaplasie kann deshalb als eine kompensatorische Erscheinung in diesen Fällen nur wenig Bedeutung gehabt haben. Wegen des äußerst spärlichen Auftretens von Myelocyten und Normoblasten im zirkulierenden Blut ist zu vermuten, daß das myeloide Gewebe in der Milz autochthonen Ursprungs ist.

8. In vielen der in der Milz in diesen Fällen gefundenen Normoblasten finden sich Bilder, die nur als eine aktive Auswanderung des Kerns aus dem Zellleib gedeutet werden können. Die Umwandlung des Erythroblasten in den Erythrocyten durch Auswanderung des Kernes muß daher als ein physiologischer Prozeß betrachtet werden.

Am Schlusse möchte ich den Herren Doktoren Winternitz und Whipple für ihre liebenswürdige Unterstützung verbindlichst danken.

Literaturverzeichnis.

1) Aubertin u. Beaujard, Actions des Rayons X sur le sang et les organes haematopoétiques. Comptes rendus de la Soc. de Biol. Séance 4. Févr. 1905.
2) Bloch, E., Beiträge zur Haematologie, Zeitschr. f. klin. Med. Bd. 43 p. 420 1901.
3) Blumenthal u. Morawitz, Experim. Untersuchungen über posthaemorrhagische Anämien, Deutsches Archiv f. klin. Med. Bd. 92 1908.
4) Bunting. Experimental anaemias in the rabbit, Journ. of exp. med. Vol. VIII p. 625 1906.
5) —, Effects of lymphotoxins and myelotoxins on the leucocytes of the blood and blood forming organs, Univ. of Penn. Med. Bull. Vol. XVI p. 200 1903.
6) Butterfield, Meyer u. Heineke, Fol. haem. Bd. VIII 1909.
7) Butterfield, Deutsches Archiv f. klin. Med. Bd. 92 p. 336.

Digitized by Google

8) Czermack, Entwicklung, Zusammensetzung und Funktion der Lymphknoten der Darmwand, Archiv f. mik. Anat. Bd. 42 p. 581 1893.
9) Dantschakoff, Untersuchungen über die Entwicklung des Blutes und Bindegewebes bei den Vögeln, Anat. Hefte Bd. XXXVII p. 113.
10) —, Untersuchungen über die Entwicklung des Blutes usw., Archiv f. mik. Anat. Bd. 73 p. 117 1909.
11) Domarus, Über Blutbildung in Milz und Leber bei exp. Anämien, Archiv f. exp. Path. u. Pharmak. Bd. LVIII p. 319 1907.
12) Dominici, Considerations générales sur la structure des appareils haematopoétiques du lapin, Compt. rendus de la soc. de biol. p. 13 1900.
13) Dominici, Sur le plan de structure du système haematopoétique, Archiv. gin. de Med. 1906 I 1 197 I Année 83 p. 641.
14) Ehrlich, Lazarus u. Pinkus, Nothnagel, Spez. Pathologie u. Therapie, Vol. VIII 1. Aufl.
15) Giemsa, Über die Färbung von Feuchtpräparaten mit meiner Azureosinmethode, Deutsches med. Wochenbl. p. 1751 Okt. 7 1909.
16) Grawitz, Klinische Pathologie des Blutes 3. Aufl.
17) Gruber, G. B., Beziehung der Milz und Knochenmark zueinander, Archiv f. exp. Path. u. Pharmak. Bd. 58 p. 289 1907.
18) Heineke, Experimentelle Untersuchung über die Einwirkung der Röntgenstrahlung auf innere Organe, Mitt. aus d. Grenzgebieten der Med. u. Chir. Bd. XIV p. 21 1905.
19) —, Exp. Untersuchungen über d. Einw. d. Röntgenstrahlungen auf d. Knochenmark, Deutsche Zeitschr. f. Chir. Bd. 78 p. 196 1905.
20) Helber u. Linser, Münch. med. Wochenschr. Bd. XV 1905.
21) Hirschfeld, H., Folia haem. Vol. III 1906.
22) —, Über einen Fall schwerer haemorrh. Diathese mit Knochenmarkatrophie, Fol. haem. Vol. III p. 429 1906.
23) Howell, W. H., The life history of the formed elements of the blood etc., Jour. of morph. IV 1891.
24) Israel u. Pappenheim, Über die Entkernung der Säugetiererythroblasten, Virchow's Archiv Bd. 143 p. 419 1896.
25) Kölliker, Über die Blutkörperchen eines menschlichen Embryos usw., Zeitschr. f. rat. Med. Bd. IV 1846.
26) Langlois et Desbouis, L'action des vapeurs de Benzol sur le sang, Hygiene gen. et appliqué, Paris II, 149 1907.
27) —, Des effets des vapeurs hydrocarbonées sur le sang, Jour. de Phys. et Path. gen. T IX p. 253 1907.
28) Lobenhoffer, Über extravaskuläre Erythropoese in der Leber, Beitr. z. path. Anat. u. allg. Path. Bd. 43 p. 125 1908.
29) Maximow, Entzündliche Neubildung von Bindegewebe, Ziegler's Beiträge Spplbd. 5 1902.
30) —, Postfötale Histogenese des myeloid. Gewebes, Beiträge z. path. Anat. u. allg. Path. Vol. 41 1907.
31) —, Untersuchungen über Blut und Bindegewebe, Archiv f. mik. Anat. Bd. 73 p. 443 1909.
32) —. Untersuchungen über Blut u. Bindegewebe, Archiv f. mik. Anat. Bd. 76 1910.
33) —, Lymphocyt als gemeinsame Stammzelle, Fol. haem. Bd. VIII 1909.
34) Meyer u. Heineke, Blutbildung bei schweren Anämien u. Leukämien, Deutsches Archiv f. klin. Med. Bd. 88 p. 435 1907.
35) —, Über Blutbildung in Milz und Leber bei schweren Anämien, Verhandlg. d. deutschen Pathol. Ges. IX p. 224 1906.
36) Morawitz u. Rehn, Über einige Wechselbeziehungen d. Gewebe in d. blutbildenden Organen, Deutsches Archiv f. klin. Med. Bd. 92 p. 109 1908.
37) Morris, Blood formation in liver and spleen in Exp. Anaemias, Bull of the John Hopkins Hosp., Vol. XVIII p. 198 1907.
38) Naegeli, Blutkrankheiten und Blutdiagnostik, Leipzig 1908.
39) Nakanishi, Vorläufige Mitteilungen über eine neue Färbungsmethode usw., Münch. med. Wochenschr. Vol. 47 p. 187 u. 680 1900.
40) Neumann, E., Über die Bedeutung des Knochenmarks für die Blutbildung usw., Archiv f. Heilk. Bd. X 1869.
41) —. Neue Beiträge zur Kenntnis der Blutbildung, Archiv f. Heilk. Bd. XV 1874.
42) Pappenheim, Neuere Streitfragen aus dem Gebiet d. Hämatologie, Zeitschr. f. klin. Med. XLVII p. 216 1902.

Digitized by Google

43) Rindfleisch, G. E., Über Knochenmark und Blutbildung, Archiv f. mik. Anat. Bd. 17 1880.
44) Santesson, Archiv. f. Hygiene Bd. XXI p. 336 1897.
45) Saxer, Entwicklung u. Bau normaler Lymphdrüsen, Anat. Hefte Bd. VI 6 I 1896.
46) Schridde, H., Über Regeneration des Blutes unter normalen und krankhaften Verhältnissen, Zentralbl. f. allgem. Path. u. path. Anat. Bd. XIX p. 865 1908.
47) —. Beiträge z. Lehre d. Zellkörnelung, Die Körnelung d. Plasmazellen, Anat. Hefte I, Abt. 85/86 Bd. 28 p. 691 1905.
48) Selling, Purpura haemorrhagica due to Benzol poisoning, Bull. of the Johns Hopkins Hosp. Vol. XXI 1910.
49) Sternberg, C., Exp. Untersuchung über die Entstehung der myeloid. Metaplasie, Beiträge z. allgem. Path. u. path. Anat. Bd. 46 p. 586 1909.
50) —, Über das Vorkommen von einkernigen, neutrophil gekörnten granulierten Leukocyten i. d. Milz, Zentralbl. f. Allgem. Path. und path. Anat. XVI 929 1905.
51) Tallquist u. Willenbrand, Zur Morphologie der weißen Blutkörperchen des Hundes u. Kaninchens, Skand. Archiv f. Phys. Bd. X 1900.
52) Thoma, Lehrbuch d. path. Anatomie Vol. I p. 393 1894.
53) Türk, Über die Regeneration des Blutes unter normalen und pathologischen Verhältnissen, Zentralbl. f. allgem. Path. u. path. Anatomie, Vol. XIX, p. 913 1908.
54) Weidenreich, Das Gefäßsystem der menschlichen Milz, Archiv f. mikr. Anat., Vol. 58 p. 247 1901.
55) —, Die roten Blutkörperchen. II. Erg. d. Anat. u. Entwicklungsgesch. Bd. XIV 1904.
56) Wolff, Über die Bedeutung der Lymphoidzelle bei der normalen Blutbildung und bei Leukämien. Zeitschr. f. klin. Med. 45, p. 385 1902.
57) Thayer, W. S., Amoebic Movements in Megaloblasts, Archiv of Int. medecine Feb. 1911.

Erklärung der Abbildungen.

Tafel I.

Die Mikrophotogramme (Fig. 1—17) sind mit dem Mikrophotographischen Apparat von Zeiß im Pathologischen Institut zu Leipzig angefertigt.

Fig. 1. Normales Knochenmark des Kaninchens. Im unteren Teil des Bildes sieht man das Fettgewebe in der Gegend der Zentralsinus. Vergr. 93fach.

Fig. 2. Ausgeprägte Aplasie des Marks nach der sechsten Benzolinjektion. Die Zahl der Parenchymzellen ist stark reduziert; sie treten einzeln und in kleinen Gruppen auf. Viele sind degeneriert. Ausgesprochenes Ödem des Reticulums und Deformierung der Fettzellen.

Fig. 3. Vorgeschrittene Aplasie des Marks nach neun Injektionen. Ausgesprochenes Ödem und Hyperämie. Vergr. 93fach.

Fig. 4. Beginnende Hyperplasie des Marks nach der zweiten Injektion. Die Parenchymelemente sind in unregelmäßigen Gruppen angeordnet. Um den Zentralsinus herum findet man große Zellgruppen. Vergr. 93fach.

Die Figuren 1—4 sind alle bei derselben Vergrößerung und an einander entsprechenden Stellen aufgenommen und zwar so, daß ein Teil des Zentralsinus eingeschlossen ist. Sie können daher direkt miteinander verglichen werden.

Fig. 5. Ein regenerierendes Mark im Frühstadium mit Erythroblasteninseln (a) und Granulocyteninseln (b); c ein Myeloblast; d Polyblasten. Vergr. 430fach.

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Fig. 6. Früh regenerierendes Mark mit Normoblasteninseln in einem sonst aplastischen Reticulum. Vergr. 175 fach.

Fig. 7. Späteres Regenerationsstadium mit großen Inseln, die lediglich aus Granulocyten bestehen. Die Inseln sind durch Flächen aplastischen Reticulums voneinander getrennt. Ödem des Reticulums. Vergr. 93 fach.

Fig. 8. Spätstadium der Regeneration mit langen Strängen von Zellen, die dem Verlnuf des Kapillaren entlang gegen die Zentralsinus hin wachsen. Die Parenchymzellen sind extravaskulär. Rechts Erythroblasten; links Granulocyten. Vergr. 175 fach.

Tafel II.

Fig. 9. Normaler Appendix des Kaninchens. a, b, c, d stellen verschiedene Teile des lymphadenoiden Gewebes der Submukosa dar. e) Gruppen mononukleärer pigmentierter Zellen. Vergr. 93 fach.

Fig. 10. Partielle Aplasie des lymphadenoiden Gewebes des Appendix. Die Lymphocyten sind fast völlig aus der Gegend a verschwunden. An ihrer Stelle finden sich große mononukleäre, pigmentierte und nichtpigmentierte Zellen. Die Submukosa ist auf etwa die Hälfte ihrer normalen Dicke reduziert (vgl. Fig. 9). Vergr. 93 fach.

Fig. 11. Extreme Aplasie des lymphadenoiden Gewebes des Appendix. x stellt alles dar, was von der Submukosa zurückgeblieben ist. Sie enthält hauptsächlich große mononukleäre, pigmentierte und nichtpigmentierte Zellen und einige wenige Lymphocyten. Neun Benzolinjektionen. Vergr. 93 fach.

Fig. 12. Normal erscheinende Lymphdrüse. Der Schnitt stammt von einem Tiere, welchem nach neun Benzolinjektionen 15 Erholungstage gewährt worden waren. Vergr. 93 fach.

Fig. 13. Lymphdrüse mit vorgeschrittener Aplasie nach acht Benzolinjektionen. Im oberen Teil des Schnitts sind so gut wie alle Lymphocyten aus dem Follikel verschwunden und nur das Stützgewebe ist zurückgeblieben. Vergr. 93 fach.

Fig. 14. Vorgeschrittene Aplasie einer Lymphdrüse (von demselben Fall wie 13). Ödem der Kapsel, Dilatation der Lymphräume, starker Verlust an Lymphocyten. Vgl. Fig. 12. — Vergr. 93 fach.

Fig. 15. Normal erscheinende Milz mit MALPIGHI'schen Körperchen, Pulpasträngen und Sinus. Der Schnitt entstammt einem Fall, bei welchem nach fünf Injektionen 19 Tage für die Regeneration zur Verfügung gestanden hatten. Vergr. 93 fach.

Fig. 16. Vorgeschrittene Aplasie der Milz nach sechs Benzolinjektionen. Im MALPIGHI'schen Körperchen ist die Zahl der Lymphocyten stark reduziert und das Stützgewebe tritt hervor. Die Pulpastränge sind dünn, kollabiert und fast vollständig frei von Parenchymzellen. Vergr. 93 fach.

Fig. 17. Dieselbe Milz bei stärkerer Vergrößerung. Man sieht die dünnen, kollabierten Pulpastränge. Vergr. 175 fach.

Tafel III.

Fig. 18. Regenerierendes Mark mit einer isolierten Gruppe von Granulocyten. a) Amphophile Myelocyten. e) Große Lymphocyten (Myeloblasten). d) Ein isolierter großer Lymphocyt. Leitz, Okular 3, Objektiv $^1/_{12}$, Ölimmersion.

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Fig. 15. Regenerierendes Mark mit Gruppen von Erythroblasten. a) ältere, b) jüngere Zellen.

Fig. 20. Lymphdrüse nach einer Benzolinjektion. a) kleiner Lymphocyt; b) großer Lymphocyt; c) Chromatinfragmente; d) Phagocyten mit Chromatinfragmenten; f) großer Lymphocyt, dessen Kern beginnende Karyolyse zeigt. Leitz, Okular 1, Objekt. $^1/_{12}$; Ölimmersion.

Fig. 21. Milz. Versuch 44. a) Milzsinus; b) Pulpastrang; c) kernhaltige rote Blutzelle mit sanduhrförmig ausgezogenem Kern. Das Protoplasma liegt mit einem Teile des Kerns im Sinus. Ein dünner Chromatinstrang geht von ihm aus durch die Sinuswand hindurch und endet als zwiebelförmige Chromatinmasse, die im Pulpastrang liegt; d) Endothelzellen; f) nackter Kern, welcher im Pulpastrang liegt. Leitz, Okular 3, Objekt. $^1/_{12}$, Ölimmersion.

Fig. 22. Milz. Versuch 44. Die Buchstaben bezeichnen dieselben Strukturen wie in XXI. Bei den Sanduhrnormoblasten c liegt mehr Kernchromatin im Pulpastrang und weniger innerhalb des Zellleibs im Sinus. e) Pigmentzelle im Sinus; k) freies Pigment im Pulpastrang. Leitz, Okular 3. Objekt. $^1/_{12}$, Ölimmersion.

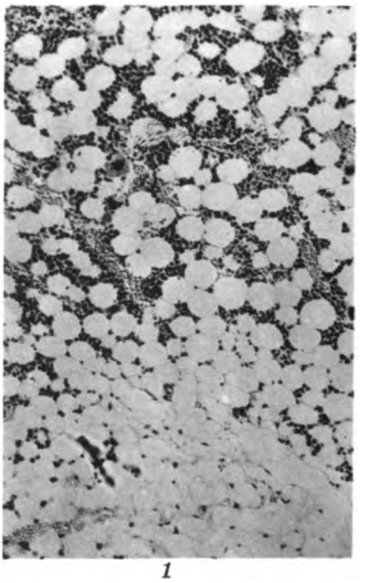
Fig. 23. Milz. Buchstaben wie in 21 und 22. Bei dem Normablasten c liegt fast der ganze Kern extrazellulär und innerhalb des Pulpastranges; nur ein kleiner Chromatinfleck ist im Zellleib geblieben. g) eosinophiler Myelocyt; h) amphophiler Myelocyt. Leitz, Okular 3, Objekt. $^1/_{12}$, Ölimmersion.

Fig. 24. Diapedese kernhaltiger roter Blutzellen in einem Blutgefäß der Froschzunge infolge von (experimenteller) Stase. Der extravaskuläre Teil der Zelle besteht aus Protoplasma a. Den von einer wohl definierten Protoplasmazone umgebenen Kern sieht man innerhalb des Gefäßes. Zu vergleichen mit c der drei vorhergehenden Figuren. Leitz, Okular 3, Objekt. $^1/_{12}$, Ölimmersion.

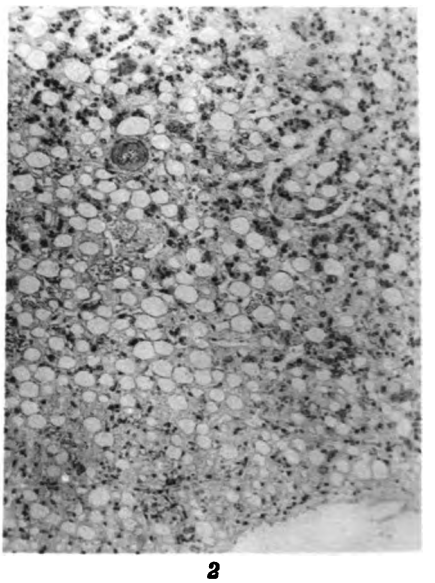
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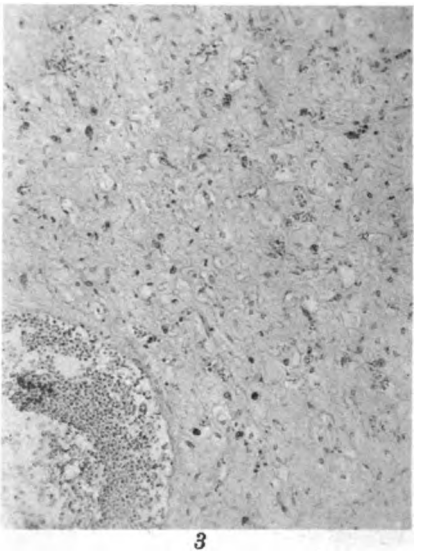
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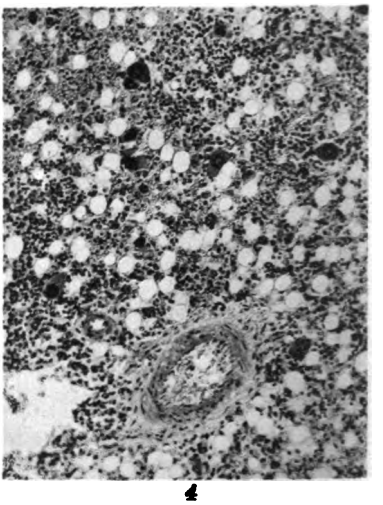
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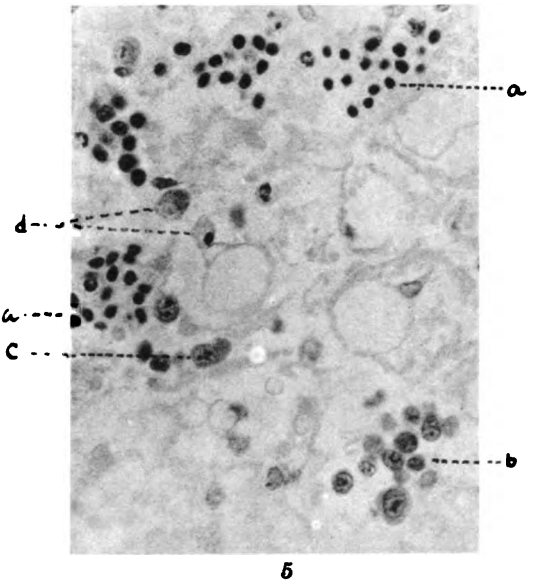


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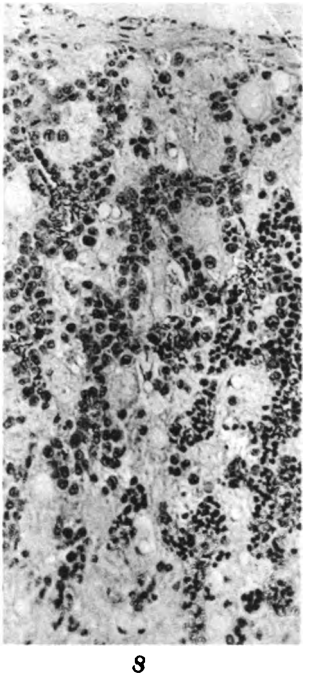


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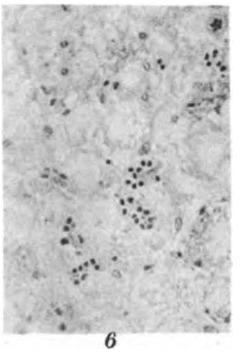
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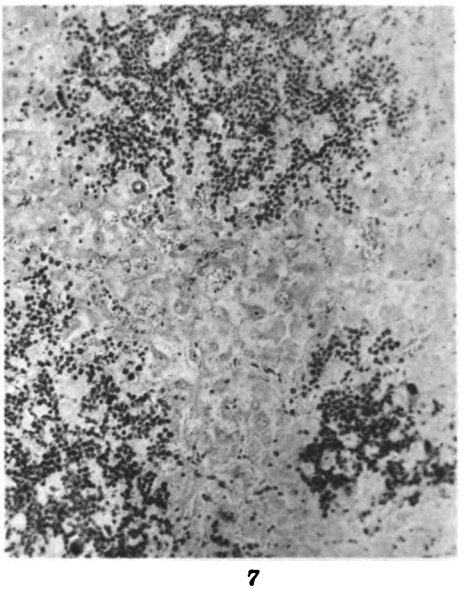
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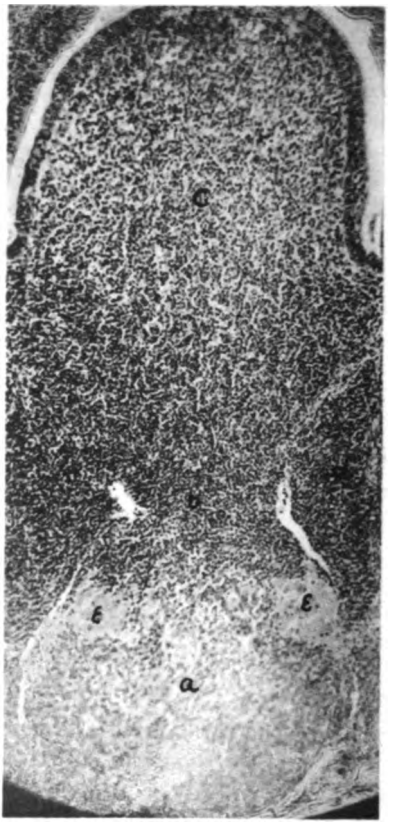
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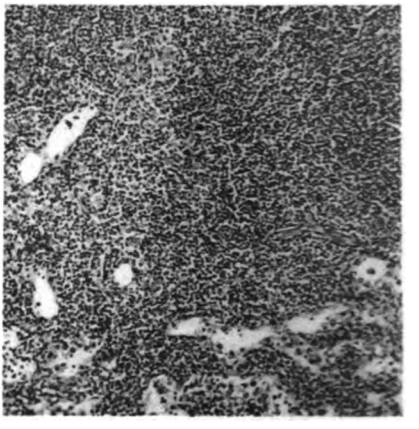
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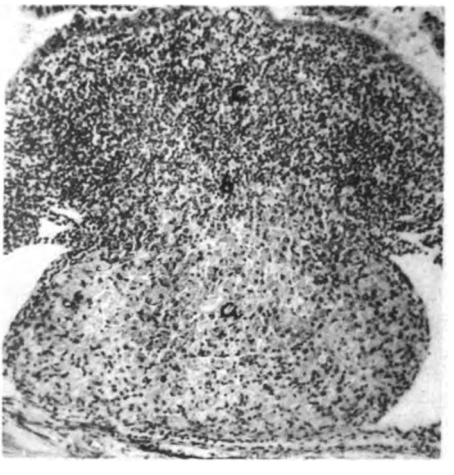
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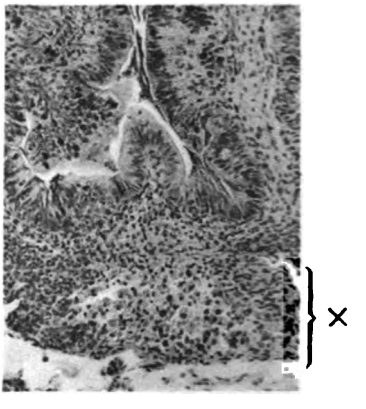
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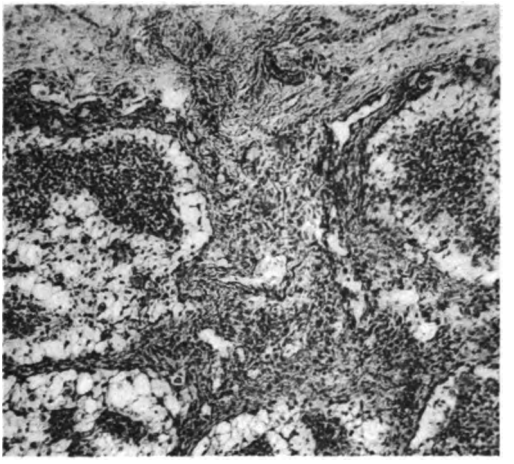


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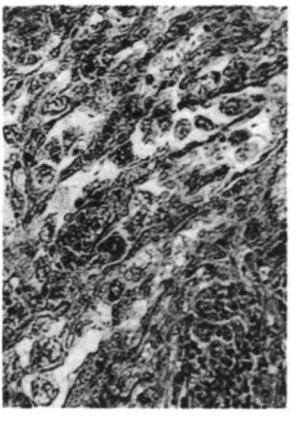


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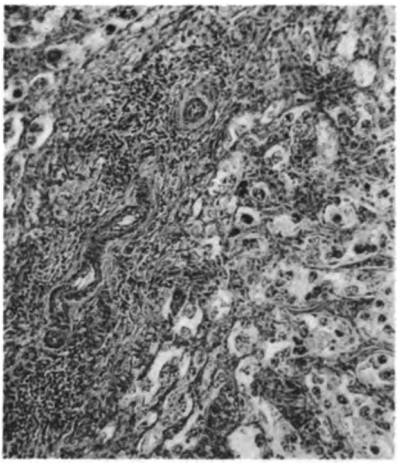
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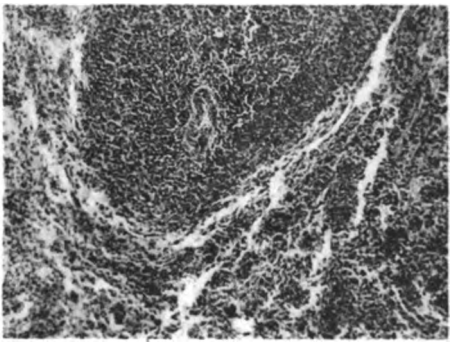
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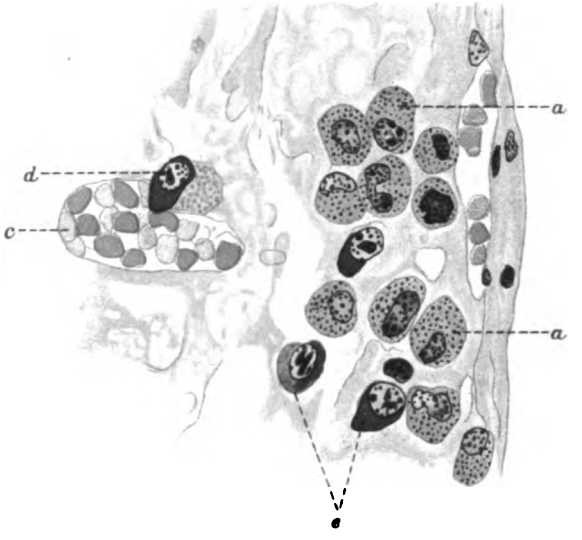
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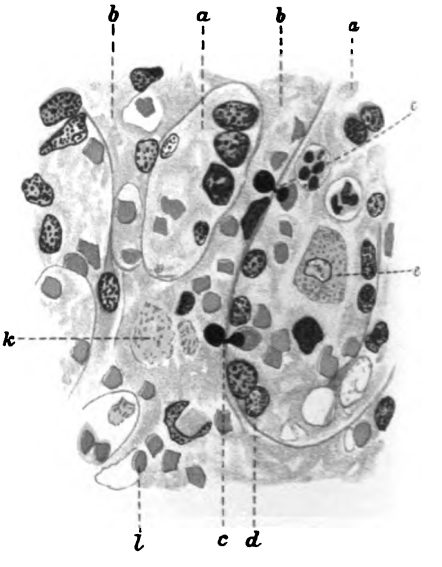
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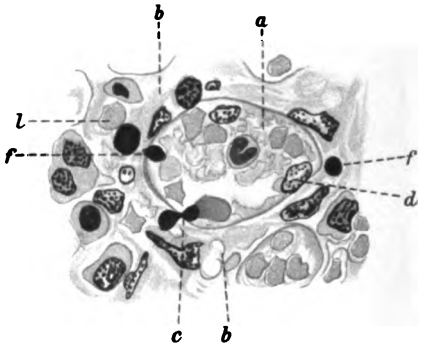




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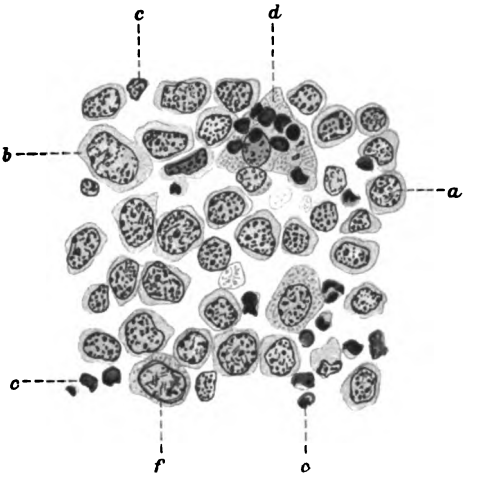
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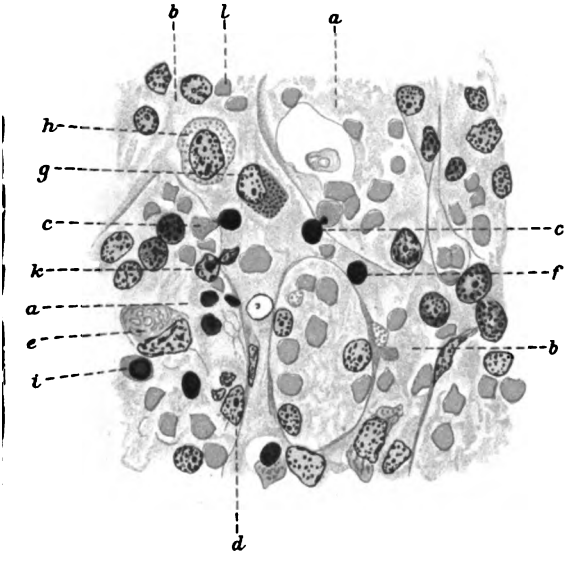
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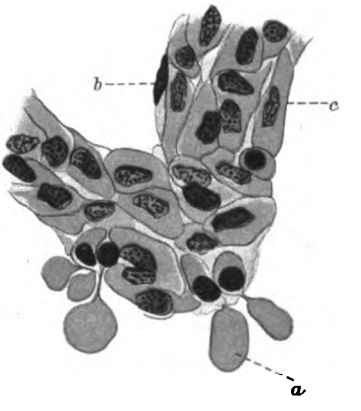
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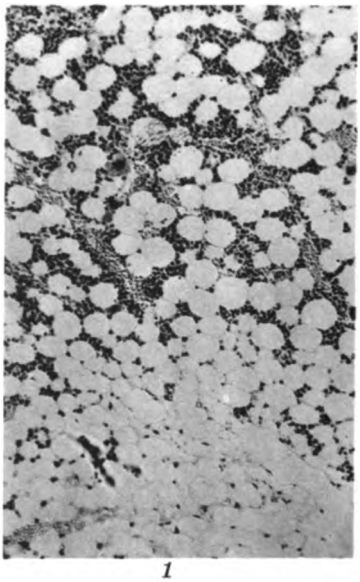
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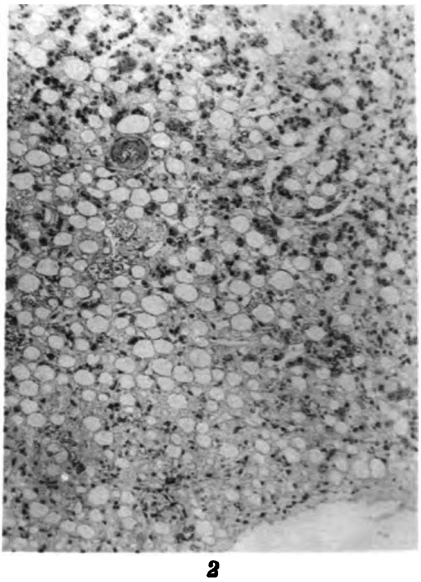
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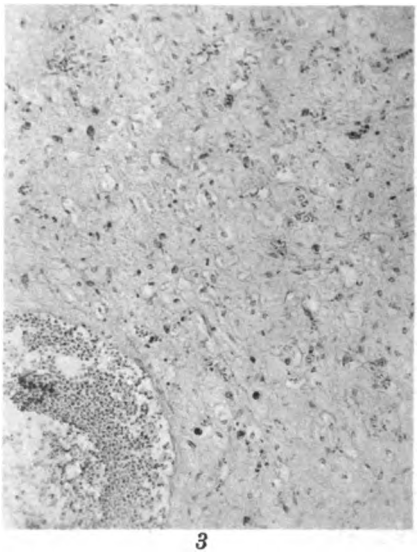
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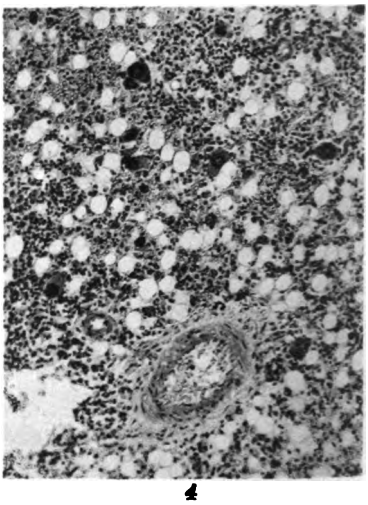
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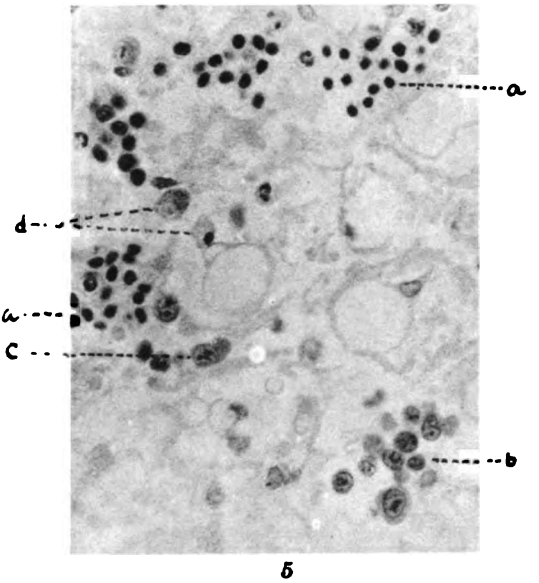


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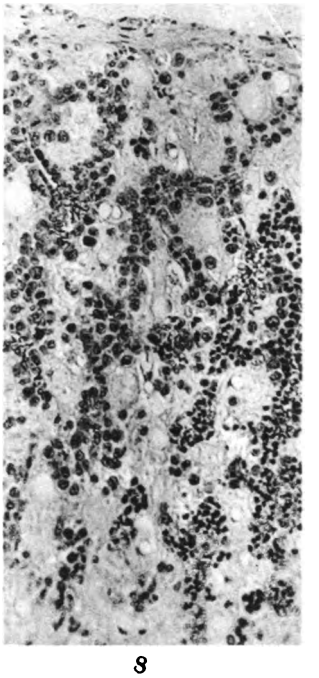


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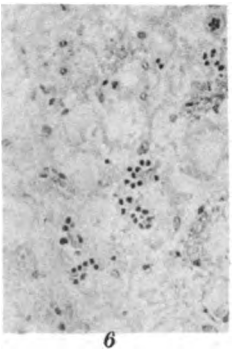
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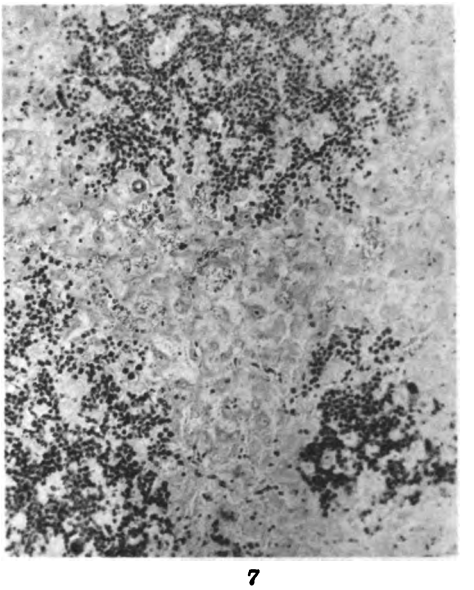
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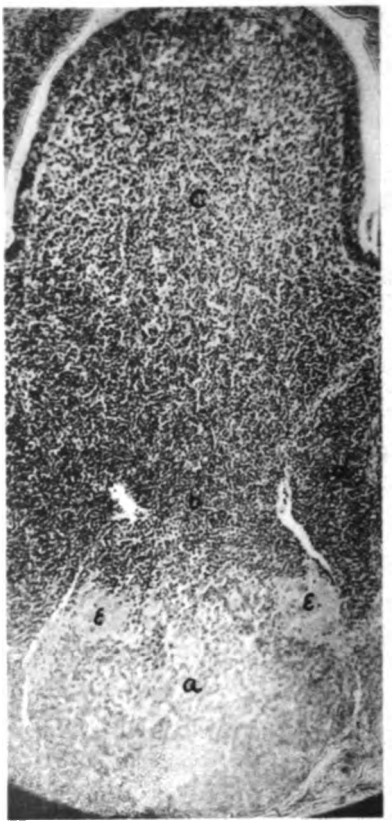
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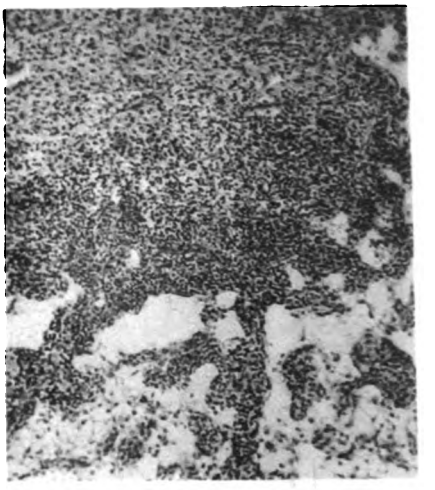
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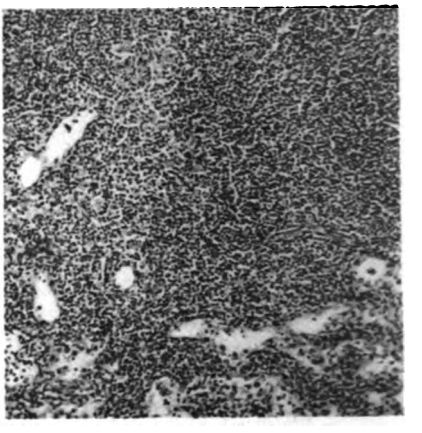
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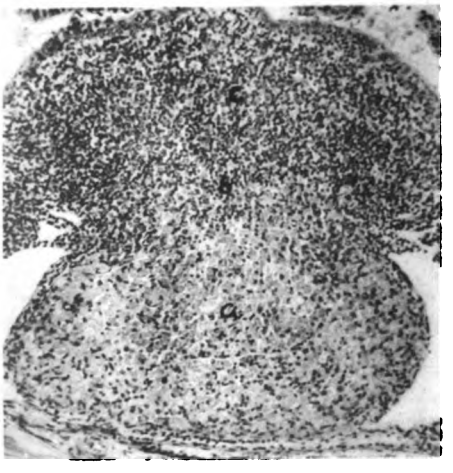
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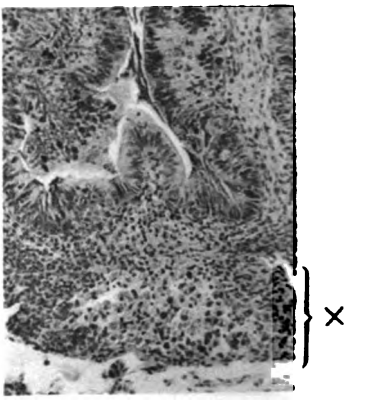
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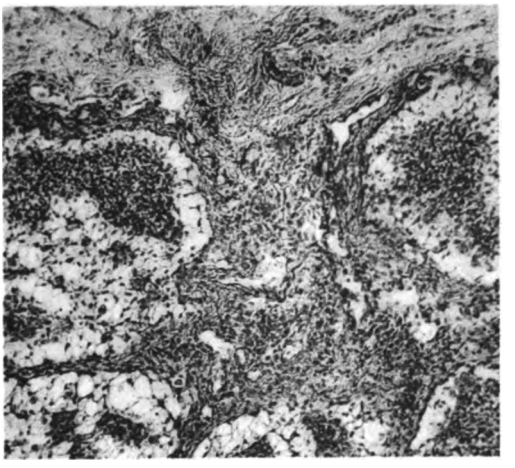


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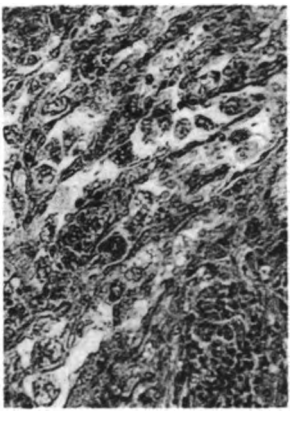


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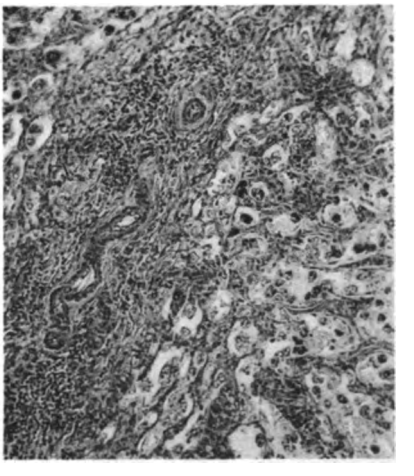
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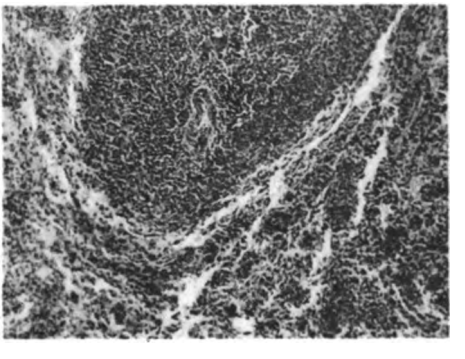
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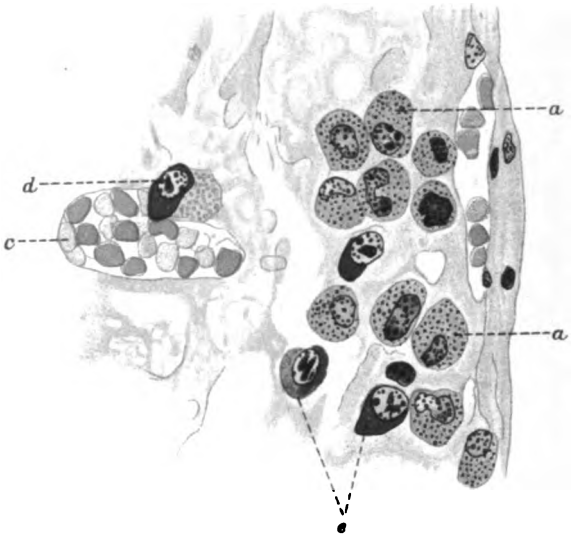
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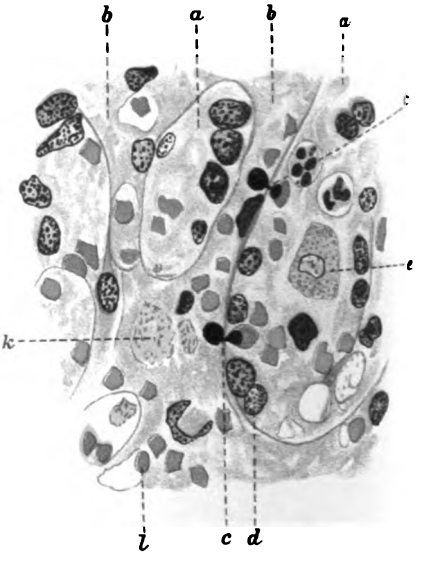
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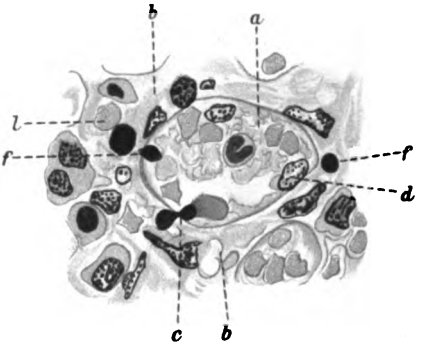




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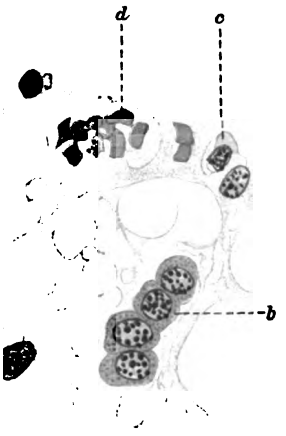
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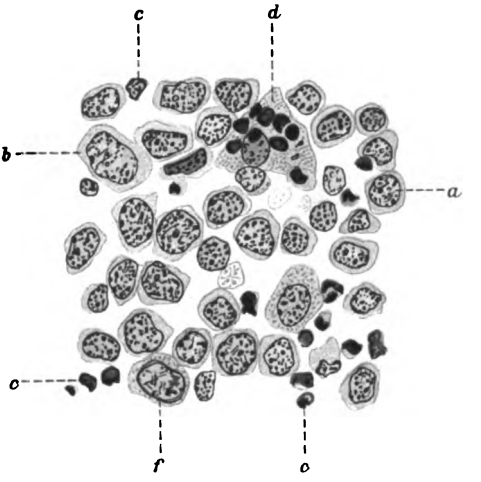
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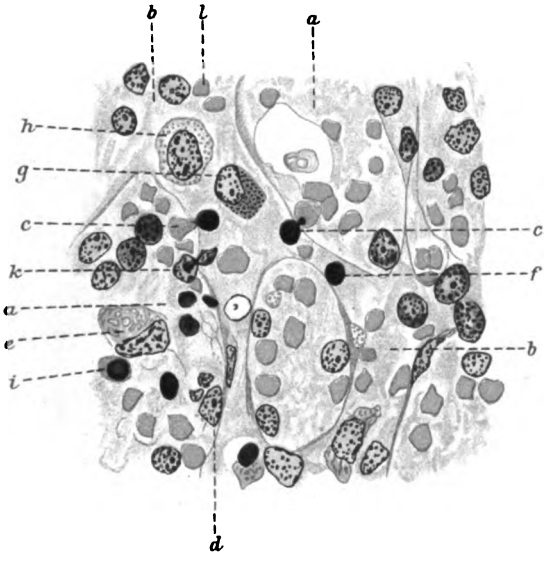
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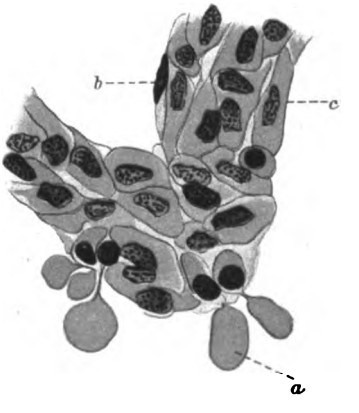
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Die Beeinflussung der Entwicklung und der Oxydationsvorgänge im Seeigelei (Arbacia) durch Basen.

Von

Jacques Loeb und Hardolph Wasteneys.

(Aus dem Rockefeller Institute, New York.)

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I.

In einer früheren Arbeit hatte Loeb[1]) gezeigt, daß die Entwicklung der frisch befruchteten Eier des Seeigels Strongylocentrotus purpuratus in einer eigentümlichen Weise von der Konzentration der Hydroxylionen der Lösung abhängt, in der die Entwicklung stattfindet. Bringt man die Eier unmittelbar nach der Befruchtung in 50 ccm einer $^{m}/_{2}$ Lösung von NaCl, KCl, $CaCl_2$ und $MgCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser enthalten sind, und setzt man zu einer solchen Lösung 0, 0,1, 0,2, 0,4, 0,8 und 1,6 ccm $^{n}/_{10}$-KHO zu, so tritt in der neutralen Lösung keine Furchung der Eier ein; und dasselbe beobachtet man auch in den Lösungen, denen man 0,8 ccm oder mehr $^{n}/_{10}$-KHO zusetzt. In den Lösungen aber, die 0,1 oder 0,2 ccm $^{n}/_{10}$-NaHO enthalten, tritt eine normale Furchung und Entwicklung der Eier zu schwimmenden Larven ein. Eine zu niedrige und eine zu hohe Konzentration der Hydroxylionen hemmt also die Furchung und Entwicklung der frisch befruchteten Eier von Strongylocentrotus purpuratus. Die minimale Konzentration der HO-Ionen, in der die Entwicklung der frisch befruchteten Eier stattfinden kann, ist etwa 10^{-6} n. Das Gesagte gilt nur für die Eier unmittelbar nach der Befruchtung. Ist die Entwicklung einmal im Gange, so kann sich das Ei eines Seeigels auch in einer neutralen Lösung der erwähnten Salze weiter entwickeln.

[1]) J. Loeb, diese Zeitschr. 2, 91, 1906.

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Loeb erklärte die Bedeutung der Hydroxylionen für die Entwicklung durch die Annahme, daß sie die Oxydationen beschleunigen. Für diese Annahme hatte sich eine Reihe von Anhaltspunkten ergeben, auf die wir hier nicht einzugehen brauchen.

O. Warburg[1]) verglich den Sauerstoffverbrauch der Eier von Strongylocentrotus in Neapel bei drei verschiedenen Konzentrationen von HO-Ionen, nämlich 10^{-8}, 10^{-6} und 10^{-3} n. Der Sauerstoffverbrauch stand im Verhältnis von 1,4 : 3,9 : 8,1. Nur bei der HO-Konzentration von 10^{-6} fand in diesen Fällen Furchung und Entwicklung statt, was den früheren Beobachtungen von Loeb entspricht.

Wir haben in der vorigen Arbeit die Untersuchung der Frage begonnen, ob die Oxydationsvorgänge die unabhängige Variable bei den Lebenserscheinungen sind. Es schien wünschenswert, für eine größere Zahl von Konzentrationen der Hydroxylionen den Zusammenhang zwischen Beeinflussung der Oxydation und der Furchung des Eies festzustellen.

Unsere Versuche wurden an den Eiern des Seeigels Arbacia in Woods Hole angestellt. Die Eier dieses Seeigels furchen und entwickeln sich schon von vornherein in einer neutralen Lösung von NaCl, KCl, $CaCl_2$ zu schwimmenden Larven und unterscheiden sich daher in diesem Punkte von den Eiern von Strongylocentrotus. Fügt man zu 100 ccm einer solchen Lösung 0,1 bis 0,2 ccm $^n/_{10}$-NaHO oder NH_4HO, so wird die Entwicklung der Eier etwas beschleunigt, und mehr Eier erreichen das Larvenstadium als in neutraler Lösung; fügt man aber 0,4 ccm oder mehr $^n/_{10}$-NaHO oder NH_4HO zu 100 ccm der Salzlösung, so tritt eine deutliche Hemmung der Entwicklung ein und die Eier leiden. Diese schädigende Wirkung des Alkalis wird um so größer, je mehr Alkali zugefügt wird.

Wir verfuhren in unseren Versuchen so, daß wir zuerst den Sauerstoffverbrauch der frisch befruchteten Eier in einer neutralen Lösung von $NaCl + KCl + CaCl_2$ bestimmten, und dann bei denselben Eiern nacheinander in zwei alkalischen Lösungen derselben Salze. Die Expositionsdauer in einer Lösung betrug $1^1/_2$ Stunden, die Temperatur 20°.

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[1]) O. Warburg, Zeitschr. f. physiol. Chem. 66, 305, 1910.

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Die in dieser Arbeit benutzten Lösungen waren stets halbgrammolekular und besaßen nach den Methoden von Friedenthal eine HO-Ionenkonzentration von 10^{-7} n, waren also genau neutral.

Die Einzelheiten der Methode der Bestimmung des O-Verbrauchs nach Winkler sind in den voraufgehenden Arbeiten gegeben.

Tabelle I gibt eine Übersicht über die Versuche und Resultate.

Tabelle I.

Versuchsreihe	Natur der Lösung	Sauerstoffverbrauch in $1^1/_2$ Std. bei 20° mg
1	100 ccm NaCl KCl $CaCl_2$ neutral	0,65
	100 „ NaCl KCl $CaCl_2$ + 0.05 ccm $^n/_{10}$-HCl . .	0,61
	100 „ NaCl KCl $CaCl_2$ + 0,1 „ $^n/_{10}$-HCl . .	0,36
2	100 ccm NaCl KCl $CaCl_2$ neutral	1,09
	100 „ NaCl KCl $CaCl_2$ + 0,21 ccm $^n/_{10}$-NaHO .	1,39
	100 „ NaCl KCl $CaCl_2$ + 0,42 „ $^n/_{10}$-NaHO .	1,94
3	100 ccm NaCl KCl $CaCl_2$ neutral	0,80
	100 „ NaCl KCl $CaCl_2$ + 0,105 ccm $^n/_{10}$-NaHO .	0,94
	100 „ NaCl KCl $CaCl_2$ + 0,21 „ $^n/_{10}$-NaHO .	1,04
4	100 ccm NaCl KCl $CaCl_2$ neutral	0,56
	100 „ NaCl KCl $CaCl_2$ + 0,42 ccm $^n/_{10}$-NaHO .	1,24
	100 „ NaCl KCl $CaCl_2$ + 0,84 „ $^n/_{10}$-NaHO .	1,53
5	100 ccm NaCl KCl $CaCl_2$ neutral	0,57
	100 „ NaCl KCl $CaCl_2$ + 0,63 ccm $^n/_{10}$-NaHO .	1,34
	100 „ NaCl KCl $CaCl_2$ + 1,25 „ $^n/_{10}$-NaHO .	1,65

Bestimmen wir nun danach den Einfluß der Konzentration der HO-Ionen auf den Sauerstoffverbrauch, indem wir den Sauerstoffverbrauch in der neutralen Lösung als 1 bezeichnen, so erhalten wir folgende Werte.

Tabelle II.

Konzentration der HO-Ionen	Koeffizient des Sauerstoffverbrauchs
$2 \cdot 10^{-10}$	0,93
10^{-7}	1,0
10^{-4}	1,17
$2{,}1 \cdot 10^{-4}$	1,30 1,27
$4{,}2 \cdot 10^{-4}$	1,78 2,21
$6{,}3 \cdot 10^{-4}$	2,35
$8{,}4 \cdot 10^{-4}$	2,74

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Zunächst muß bemerkt werden, daß die Eier von Arbacia sich selbst noch in einer schwach sauren Lösung $C_{HO} = 2 \cdot 10^{-10}$ zu furchen vermögen. Aber in $C_{HO} = 10^{-10}$ findet keine Furchung mehr statt. Die Eier agglutinieren, und die Werte für den Sauerstoffverbrauch fallen daher zu niedrig aus und sind nicht verwertet worden. Bei $C_{HO} = 10^{-7}$ findet vollständige Entwicklung der Eier bis zum Blastulastadium oder bis zur beginnenden Gastrulation statt. Bei $C_{HO} = 4 \cdot 10^{-4}$ wird die Entwicklung gehemmt resp. unterdrückt.

Der Sauerstoffverbrauch wächst nun anfangs mit zunehmender C_{HO} sehr langsam; einem Anwachsen der C_{HO} von $2 \cdot 10^{-10}$ bis zu 10^{-4} entspricht ein Anwachsen des Sauerstoffverbrauchs um rund 20 bis 30%, während einem Anwachsen der C_{HO} von 10^{-4} auf $8 \cdot 10^{-4}$ ein Anwachsen des Sauerstoffkonsums von etwas mehr als 100% entspricht. Aus diesen Versuchen dürfen wir nun zunächst den Schluß ziehen, daß die Entwicklungshemmung bei Zusatz von zuviel NaHO sicher nicht auf der hierdurch bedingten Oxydationsbeschleunigung beruht. Wir sehen nämlich, daß der Zusatz von so viel NaHO, als nötig ist, um die Oxydationen auf das Doppelte des für die Furchung nötigen Minimums zu erhöhen, bereits die Furchung unterdrückt. Unsere Versuche waren bei einer Temperatur von 20° angestellt worden. Wir sahen nun in der voraufgehenden Abhandlung, daß, wenn man die Temperatur von 20° auf 30° erhöht, die Oxydationen mehr als verdoppelt werden; daß aber eine durch diese Temperaturerhöhung bedingte Verdoppelung der Oxydationen nicht etwa die Furchung des Eies von Arbacia hemmt, sondern im Gegenteil dieselbe bis auf nahezu das Doppelte beschleunigt. Daraus folgt also mit Sicherheit, daß die Hemmung der Entwicklung durch den Zusatz von NaHO durch andere Einflüsse als durch die Oxydationsbeschleunigung bedingt ist. Es ist ja gewissermaßen selbstverständlich, daß ein Stoff wie NaHO in mehr als einen der chemischen Prozesse im Ei einzugreifen imstande ist.

II.

In früheren Arbeiten hatte Loeb gezeigt, daß der Zusatz derselben Menge von NaHO ganz verschiedene Wirkungen auf die Eier ausübt, je nach der Natur der Salzlösung, in der sich

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dieselben befinden[1]). Setzt man etwa 0,1 ccm $^n/_{10}$-NaHO zu 50 ccm einer neutralen Lösung von NaCl oder von NaCl + KCl, so erhöht man damit die Schädlichkeit der Lösung auf die frisch befruchteten Eier des Seeigels (Strongylocentrotus und Arbacia). Fügt man aber dieselbe Menge NaHO zu 50 ccm einer Lösung von NaCl + $CaCl_2$, so verringert man damit die Giftigkeit einer solchen Lösung bei Arbacia. Die Eier, die in einer neutralen Lösung von NaCl + $CaCl_2$ sonst zerfallen, bleiben viel länger am Leben, wenn man die Lösung schwach alkalisch macht. Hat man es mit einer neutralen Lösung von NaCl + KCl + $CaCl_2$ zu tun, so können sich in einer solchen Lösung die frisch befruchteten Eier von Arbacia, wie schon erwähnt, bis zu schwimmenden Larven entwickeln; der Zusatz von etwas NaHO erhöht die Zahl und Lebensdauer der sich zu Larven entwickelnden Eier; aber die entgiftende Wirkung des Zusatzes von NaHO ist nicht so scharf ausgesprochen wie beim Zusatz von NaHO zu einer Mischung von NaCl + $CaCl_2$. Wir müssen die Frage aufwerfen: Hängen diese Verschiedenheiten von der Wirkung von NaHO auf die Oxydationen ab? Wir haben nicht sehr viele Versuche angestellt, aber dieselben scheinen die Frage eindeutig in dem Sinne zu entscheiden, daß die Verschiedenheit der Wirkung derselben Konzentration von Hydroxylionen in verschiedenen Salzlösungen bei Arbacia nicht auf Unterschiede der Oxydationsbeschleunigung zurückzuführen ist.

Tabelle III.

Versuchsreihe	Natur der Lösung	Sauerstoffverbrauch der Eier bei 20° in $1^1/_2$ Std. mg
1	100 ccm NaCl + $CaCl_2$ neutral	0,65
	100 „ NaCl + $CaCl_2$ + 0,1 ccm $^n/_{10}$-NaHO .	0,82
	100 „ NaCl + $CaCl_2$ + 0,2 „ $^n/_{10}$-NaHO .	1,02
2	100 ccm NaCl + $CaCl_2$ neutral	0,67
	100 „ NaCl + $CaCl_2$ + 0,4 ccm $^n/_{10}$-NaHO .	1,95
	100 „ NaCl + $CaCl_2$ + 0,8 „ $^n/_{10}$-NaHO .	1,33

Der Zusatz von 0,8 ccm NaHO zu 50 ccm NaCl + $CaCl_2$ schädigte die Eier bereits so stark, daß eine Abnahme der Oxydation eintrat.

[1]) Loeb, diese Zeitschr. 2, 89, 1906.

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Bestimmen wir den Geschwindigkeitskoeffizienten für die Oxydation für die verschiedenen Konzentrationen von NaHO in diesem Falle (indem wir die Geschwindigkeit des O-Verbrauchs in der neutralen Lösung von $NaCl + CaCl_2 = 1$ setzen, (so erhalten wir:

Tabelle IV.

$NaCl + CaCl_2$ (neutral)	$C_{HO} = 10^{-7}$ n	1,0
	$C_{HO} = 10^{-4}$ n	1,26
	$C_{HO} = 2 \cdot 10^{-4}$ n	1,57
	$C_{HO} = 4 \cdot 10^{-4}$ n	2,9

Diese Koeffizienten der Oxydationsbeschleunigung sind nahezu identisch mit denjenigen, die wir für die Oxydationsbeschleunigung durch NaHO in einer Lösung von $NaCl + KCl + CaCl_2$ fanden. Wir müssen daraus schließen, daß die „entgiftende" Wirkung des Zusatzes einer kleinen Menge von NaHO zu einer Mischung von $NaCl + CaCl_2$, die bei den frisch befruchteten Eiern von Arbacia beobachtet wird, nicht auf Beeinflussung der Oxydation zurückgeführt werden kann. Vielmehr müssen wir annehmen, daß in diesem Falle eine Spur von NaHO eine ähnliche Wirkung ausübt, wie sie auch dem Zusatz von K zukommt, d. h. daß es sich um eine physikalisch-chemische Wirkung auf die Kolloide der Zelloberfläche handelt, um eine Art „Gerbwirkung".

Was endlich die direkt schädliche cytolysierende Wirkung kleiner Mengen von NaHO betrifft, die man beobachtet, wenn man dieselbe zu einer Mischung von $NaCl + KCl$ zusetzt, so darf dieselbe wohl ebenfalls nicht auf die Oxydationsbeschleunigung durch NaHO zurückgeführt werden, sondern dieselbe muß auf einer anderen Wirkung von NaHO beruhen, wie der folgende Versuch zu beweisen scheint.

Tabelle V.

Natur der Lösung	Sauerstoffverbrauch der Eier in $1^1/_2$ Std. bei 20° mg
100 ccm $NaCl + KCl$ neutral	0,79
100 „ $NaCl + KCl + 0{,}1$ ccm $^n/_{10}$-NaHO	1,13
100 „ $NaCl + KCl + 0{,}2$ „ $^n/_{10}$-NaHO	1,17

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In einer neutralen Lösung von NaCl + KCl furchen sich alle Eier bis zum 64. Zellstadium; in einer Lösung mit 0,2 ccm $^{n}/_{10}$-NaHO erreichten nur wenige Eier das Zweizellenstadium, bei den meisten tritt schon vor der Furchung die Cytolyse ein. Das erklärt wohl die geringe Zunahme der Oxydationen in dieser Lösung im Vergleich mit der voraufgehenden Lösung, bei der 0,1 ccm $^{n}/_{10}$-NaHO zugesetzt war. In der letzteren Lösung furchen sich alle Eier.

Sollte eine Wiederholung dieses Versuchs bei den Eiern von Arbacia diese Zahlen bestätigen, so würde daraus folgen, daß der Unterschied in der Wirkung von NaHO, je nachdem es zu einer Lösung von NaCl + KCl + $CaCl_2$ oder von NaCl + $CaCl_2$ oder zu einer Lösung von NaCl + KCl zugesetzt wird, nicht auf Unterschiede in der Oxydationsbeeinflussung durch NaHO bezogen werden darf. Denn bei dem Zusatz von 0,1 ccm $^{n}/_{10}$-NaHO zu einer neutralen Mischung von NaCl + $CaCl_2$ wurde die Oxydationsgeschwindigkeit um 26%, bei Zusatz derselben Menge zu einer Mischung von NaCl + KCl um 46% erhöht. Da die Versuche bei einer Temperatur von 20° angestellt worden waren, so entspricht der Unterschied in der Oxydationsgeschwindigkeit einem Temperaturunterschied von weniger als 10°. Wie wir aber in der früheren Arbeit sahen, wirkt eine Temperaturerhöhung von 20° auf 30° auf das Ei von Arbacia nicht schädigend, sondern geradezu entwicklungsbeschleunigend.

III.

Im Anschluß an frühere Versuche von O. Warburg[1]) ist O. Meyerhof[2]) neuerdings zu folgendem Schluß gelangt: „Danach wird die normale Atmungsgröße der Strongylocentrotus-Eier (für nicht zu lange Zeiten) ausschließlich durch 3 Komponenten bestimmt: NaCl, Ca und OH. NaCl allein gibt den 4 bis 5fachen Wert gegenüber der wirklich stattfindenden Atmung, Ca drückt ihn auf das 12 oder 15fache herunter und OH′ steigert ihn dann wieder auf das 3fache, woraus die normale Atmung resultiert.“ Und weiterhin: „Der Einfluß von K auf die Atmung in NaCl ist fast Null.“

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[1]) Warburg, Zeitschr. f. physiol. Chem. 66, 305, 1910.

[2]) Meyerhof, diese Zeitschr. 33, 291, 1911.

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Wir haben keine Versuche über die Atmung der Eier von Strongylocentrotus angestellt, weil uns dieselben hier nicht zur Verfügung stehen. Dagegen haben wir schon im vorigen Jahre an den Eiern von Arbacia Versuche angestellt, die nichts von einer derartigen Beeinflussung der Atmung durch Salze erkennen lassen, die Meyerhof für die Eier von Strongylocentrotus gefunden hat. In einer reinen neutralen NaCl-Lösung war die Atmung nicht um das 4 bis 5fache erhöht, sondern praktisch unverändert; der Zusatz von Ca setzte die Atmung nicht auf das 12 bis 15fache herunter, sondern ließ dieselbe praktisch unverändert; und die vorliegende Arbeit zeigt, daß Erhöhung der HO-Ionenkonzentration von 10^{-7} n auf 10^{-6} n die Atmung bei Arbacia im besten Falle nur um rund 20% erhöht. Die Versuchsdauer war bei Meyerhof nur 12 Minuten. Darin liegt eine Gefahr. Es ist nämlich sehr wichtig, daß die Eier gleichmäßig suspendiert gehalten werden; findet in zwei zu vergleichenden Versuchen eine ungleiche Verteilung der Eier in der Lösung statt, beispielsweise ein rascheres Sinken der Eier je nach der Natur der Lösung oder eine größere Tendenz zum Zusammenballen oder zur Haufenbildung der Eier in der einen als in der anderen Lösung, so muß ein großer und dem Sinne nach konstanter Unterschied in dem Sauerstoffverbrauch entstehen. Dieser Fehler muß sich dann besonders bemerklich machen, wenn man mit einer exzessiven Menge von Eiern arbeitet, wie das in Meyerhofs Versuchen der Fall war, der die kurze Dauer seiner Versuche durch einen Überschuß in der Masse der Eier in jedem Versuch kompensieren mußte. Daß die Eier sich in verschiedenen Lösungen in der Tat physikalisch verschieden verhalten, geht u. a. aus der folgenden Angabe Meyerhofs hervor: „In NaCl-Lösung schrumpfen die Eier (nach anfänglicher Ausbildung) und nehmen beim Absetzen nur $^1/_3$ des Volumens am Boden der Versuchsflasche ein wie im Seewasser. Genau so verhalten sich die Eier in ($NaCl + KCl$). Umgekehrt nehmen die Eier in ($NaCl + CaCl_2$) ebensoviel Platz ein wie im Seewasser[1])." Bei Arbacia haben wir derartige Wirkungen der Lösungen nicht bemerkt.

[1]) Es sei auch noch bemerkt, daß Meyerhofs Lösungen 0,65 m betrugen, während wir mit $^m/_2$ Lösungen arbeiteten, die für Arbacia isotonisch sind.

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Da wir mit Versuchen über Salzwirkungen beschäftigt sind und da solche Schlüsse, wie Meyerhof sie zieht, für die Theorie der Salzwirkung von der größten Bedeutung sein müßten, haben wir unsere vorjährigen Versuche an Arbacia noch einmal wiederholt. Der wesentliche Punkt ist ein Vergleich der Wirkung von (NaCl + KCl) und (NaCl + $CaCl_2$) auf die Atmung der Eier von Arbacia. Ca sollte die Atmung auf das 12 bis 15fache des Wertes in Seewasser herunterdrücken; in Na + K sollte dieselbe auf das 4 bis 5fache ihres Wertes im Seewasser erhöht sein.

Wir verfuhren zunächst so, daß wir dieselben Eier $1^1/_2$ Stunden bei 20° nacheinander in (Na + K + Ca)[1]), in (Na + Ca) oder (Na + K) atmen ließen. Die Lösungen waren alle neutral. 1 Stunde lang wurde das Gefäß je einmal in 3 Minuten rotiert und für eine gleichmäßige Suspension der Eier gesorgt. Wir geben das Resultat von einer Versuchsreihe wieder.

Tabelle VI.

Natur der Lösung	O-Verbrauch in $1^1/_2$ Std. bei 20° mg
100 ccm NaCl $CaCl_2$ KCl, neutral . . .	0,58
100 „ NaCl + KCl neutral . . .	0,61
100 „ NaCl + $CaCl_2$ neutral . . .	0,28

Die Eier hatten sich in der Lösung von NaCl + KCl alle weiter gefurcht, aber in der Lösung von NaCl + $CaCl_2$ war die Furchung nicht weiter gegangen. Das bedingte wohl den Abfall im Sauerstoffverbrauch. Die Eier waren aber nicht tot; denn ein Teil derselben, der nach Abschluß des Versuchs in normales Seewasser übertragen wurde, hatte sich am nächsten Morgen zu schwimmenden Larven entwickelt. Man sieht, daß die Übertragung der Eier aus einer Lösung von NaCl + $CaCl_2$ + KCl in eine Lösung von NaCl + KCl (also ohne Ca) die Atmung völlig unverändert läßt. Es sei auch ausdrücklich bemerkt, daß die Eier nach der Übertragung in NaCl + KCl erst dreimal mit dieser Lösung gewaschen wurden, um ja sicher zu sein, daß praktisch alles Ca entfernt war, ehe mit dem Atmungsversuch in dieser Lösung begonnen wurde.

[1]) In NaCl + KCl + $CaCl_2$ ist der Sauerstoffverbrauch der Arbaciaeier ungefähr derselbe wie in Seewasser.

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Es schien uns aber doch nötig zu sein, unser Versuchsresultat noch einmal nach einer anderen Methode zu bestätigen. Wir hatten in den bisher erwähnten Versuchen immer die Atmung bei denselben Eiern nacheinander in verschiedenen Lösungen bestimmt. Warburg und Meyerhof hatten die Eier in zwei Teile geteilt und gleichzeitig die Atmung der beiden Hälften in verschiedenen Lösungen bestimmt. Wir vermieden diese Methode bisher, weil wir eine Garantie dafür vermißten, daß uns die vollkommene Halbierung der in einer Flüssigkeit suspendierten Eier gelungen sei. Ist die Suspension nicht vollkommen homogen, so kann eine Halbierung der Flüssigkeitsmenge keine Halbierung der Masse der Eier ergeben. Wir übten uns nun auf die Methode der Halbierung ein und stellten zunächst einen Kontrollversuch an, ob uns die Halbierung gelungen sei. Eine Menge von frisch befruchteten Arbaciaeiern wurde in $NaCl + KCl + CaCl_2$ gebracht und die Menge der Flüssigkeit mit den anscheinend gleichmäßig suspendierten Eiern halbiert. Der Sauerstoffverbrauch wurde in jeder Partie bei 20° für $1^1/_2$ Stunden (1 Stunde rotiert, $^1/_2$ Stunde für das Niedersetzen der Eier) bestimmt und ergab in beiden Fällen genau dieselbe Menge, nämlich 0,68 mg. Die vollständige Übereinstimmung in der zweiten Dezimale dürfte wohl ein reiner Zufall sein, aber die Übereinstimmung der Zahlen schien eine Gewähr dafür zu sein, daß wir mit der Methode weitergehen durften.

Im nächsten Versuch wurde nun die eine Hälfte frisch befruchteter Eier in $NaCl + KCl + CaCl_2$ gebracht, die zweite Hälfte in $NaCl + CaCl_2$.

Sauerstoffverbrauch:

in $1^1/_2$ Stunden bei 20° in $NaCl + KCl + CaCl_2$ (neutral) 0,74 mg,
in $1^1/_2$ „ „ 20° „ $NaCl + CaCl_2$ (neutral) 0,67 mg.

Der Sauerstoffverbrauch ist etwas geringer in $NaCl + CaCl_2$, vermutlich weil hier die Furchung nicht so weit fortschritt wie in der anderen Lösung. In $NaCl + KCl + CaCl_2$ waren alle Eier am Ende des Versuchs im 4. Zellstadium; in $NaCl + CaCl_2$ meist im 2. Zellstadium und nur ein Teil im 4. Zellstadium.

Im nächsten Versuch wurde die eine Hälfte der Eier in $NaCl + KCl + CaCl_2$ gebracht und die zweite Hälfte in

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$NaCl + KCl$. Vor Beginn des Versuchs waren die Eier dreimal in ihrer Lösung gewaschen worden.

Sauerstoffverbrauch:

in $1^1/_2$ Stunden bei 20^0 in $NaCl + KCl + CaCl_2$ (neutral) 0,65 mg,
in $1^1/_2$ „ „ 20^0 „ $NaCl + KCl$ (neutral) 0,73 mg.

Wir kommen also wieder zu demselben Resultat, das wir auch bei der anderen Methode erhielten: Bei Arbaciaeiern wird die Oxydationsgeschwindigkeit durch die Gegenwart der normalen Menge von Ca überhaupt nicht oder nur in kaum merkbarer Weise bei der Dauer unserer Versuche verringert.

Meyerhof hat mit Recht in seiner Arbeit darauf hingewiesen, daß ein von uns im vorigen Jahre mitgeteilter Versuch in einem Punkte unverständlich sei[1]). Die Unverständlichkeit rührt aber lediglich von einem Schreibfehler her, der sich in unser Protokollbuch eingeschlichen hat, was uns der Verantwortlichkeit für den Fehler natürlich nicht enthebt. Wir gaben nämlich an (S. 348), daß in $NaCl + KCl + CaCl_2$ die Eier 0,70 mg O verbrauchen, daß aber nach Zusatz von NaCN der Verbrauch auf 0,44 mg fiel. Da nun NaCN den O-Verbrauch auf ein Viertel oder mehr herunterdrückt, so muß der Wert 0,7 für die Lösung ohne NaCN auf einem Schreibfehler beruhen. Wir haben den Versuch dieses Jahr wiederholt und die folgenden Werte erhalten:

Sauerstoffverbrauch:

in $1^1/_2$ Stunden bei 20^0 in 100 ccm $NaCl + KCl + CaCl_2$ (neutral) . . . . . 0,74 mg,
in $1^1/_2$ „ „ 20^0 „ 100 ccm $NaCl + KCl + CaCl_2$ $+ 0{,}3$ ccm $^1/_{10}{}^0/_0$ NaCN . . 0,15 mg,
in $1^1/_2$ „ „ 20^0 „ 100 ccm $NaCl + 3$ ccm $^1/_{10}{}^0/_0$ NaCN . . . . . 0,26 mg.

Die Eier hatten im Versuche insofern nicht gelitten, als sie nach dem Versuche in normales Seewasser gebracht wurden und sich hier alle zu schwimmenden Larven entwickelten. Wir finden auch hier wieder ein Anwachsen des O-Verbrauchs in der cyannatriumhaltigen NaCl-Lösung. Wir wollen aber darauf kein Gewicht legen, da uns dieser Gegenstand hier

[1]) Loeb und Wasteneys, diese Zeitschr. 28, 348, 1910.

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nicht interessiert. (Es ist möglich, daß kleine Dosen NaCN in einer NaCl-Lösung die Oxydationen im Arbaciaei etwas weniger herabdrücken, als wenn die Lösung auch noch Ca enthält.)

Wir kommen deshalb zu einer Bestätigung des von uns schon im vorigen Jahr ausgesprochenen Satzes, daß der Sauerstoffverbrauch der frisch befruchteten Eier von Arbacia bei der von uns gewählten Versuchsdauer bei Gegenwart von Ca derselbe ist wie bei Abwesenheit von Ca. Die von Meyerhof bei den Eiern von Strongylocentrotus erhaltenen Resultate lassen sich bei den Eiern von Arbacia in Woods Hole nicht bestätigen.

IV.

Ist die Beschleunigung der Oxydationen und die fördernde Wirkung von NaHO auf die Furchung durch die HO-Ionen bedingt? An dieser Annahme würden wir kaum Veranlassung haben zu zweifeln, wenn nicht der eine von uns nachgewiesen hätte, daß die entwicklungsbegünstigende Wirkung von NaHO auf das Ei von Strongylocentrotus auch durch eine so schwache Base wie Neutralrot ersetzt werden kann[1]). Die Eier von Strongylocentrotus sind nicht imstande, sich in einer neutralen Lösung von $NaCl + KCl + CaCl_2 + MgCl_2$ zu entwickeln. Fügt man aber etwa 0,4 bis 0,8 ccm $^m/_{100}$ Neutralrot zu 50 ccm dieser Lösung, so entwickeln sich alle Eier zu schwimmenden Larven. Wir hielten es für angezeigt, zu untersuchen, ob die Steigerung der Oxydationen im befruchteten Ei von Arbacia durch NH_4OH von derselben Größenordnung ist, wie die durch dieselben Konzentrationen von NaHO bedingte Oxydationssteigerung. Das scheint der Fall zu sein, wie die folgenden Versuche zeigen.

Tabelle V.

Versuchsreihe	Natur der Lösung	Sauerstoffverbrauch der Eier in $1^1/_2$ Std. bei 20° mg
1	100 ccm $NaCl + KCl + CaCl_2$ neutral	1,00
	100 „ $NaCl + KCl + CaCl_2 + 0{,}2$ ccm $^n/_{10}$-NH_4OH	1,19
	100 „ $NaCl + KCl + CaCl_2 + 0{,}4$ „ $^n/_{10}$-NH_4OH	1,60
2	100 ccm $NaCl + KCl + CaCl_2$ neutral	0,56
	100 „ $NaCl + KCl + CaCl_2 + 0.4$ ccm $^n/_{10}$-NH_4HO	0,93
	100 „ $NaCl + KCl + CaCl_2 + 0{,}8$ „ $^n/_{10}$-NH_4HO	1,70

[1]) Loeb, diese Zeitschr. 26, 279, 1910.

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Bezeichnen wir den Sauerstoffverbrauch in der neutralen Lösung mit 1, so ergeben sich die folgenden Werte für die Oxydationsbeschleunigung durch verschiedene Konzentrationen von NH_4OH:

Tabelle VI.

Konzentration der NH_4OH-Lösung	Koeffizient des Sauerstoffverbrauchs
0	1,00
$2 \cdot 10^{-4}$	1,19
$4 \cdot 10^{-4}$	1,60 1,66
$8 \cdot 10^{-4}$	3,04

Ein Vergleich mit Tabelle II zeigt, daß die Oxydationsbeschleunigung durch NH_4OH von derselben Größenordnung ist, wie die Oxydationsbeschleunigung durch NaHO von derselben Konzentration.

Es ist deshalb zum mindesten fraglich, ob die oxydationsbeschleunigende Wirkung der Basen in diesem Falle auf die Konzentration der freien Hydroxylionen bezogen werden darf. Schon in einer früheren Arbeit hat Loeb darauf hingewiesen, daß hier wohl die durch die Base bewirkte Salzbildung das Wesentliche sein könnte. Auch in bezug auf die entgiftende oder „schützende" Wirkung gegenüber einer Lösung von $NaCl + CaCl_2$ verhalten sich NH_4OH und NaHO gleich, solange die gewählten Konzentrationen nicht zu hoch sind.

V.

Zusammenfassung der Ergebnisse.

1. Die Furchung und der Sauerstoffverbrauch der frisch befruchteten Eier von Arbacia werden in Lösungen von $NaCl + KCl + CaCl_2$ untersucht, in denen die Konzentration der HO-Ionen durch Zusatz von HCl resp. NaHO variiert ist. Es ergibt sich, daß dem Ansteigen der C_{HO} von 10^{-10} n auf 10^{-4} n eine Zunahme der Oxydationsgeschwindigkeit von nur etwa 20% entspricht; dann aber folgt eine raschere Zunahme der Oxydationsgeschwindigkeit mit wachsender C_{HO}. Von $C_{HO} = 10^{-4}$ n bis $C_{HO} = 8.10^{-4}$ steigt die Oxydation auf das Doppelte.

2. Die früher von Loeb und von Warburg beobachtete Tatsache, daß NaHO die Furchung hemmt, wenn es in zu

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hoher Konzentration zugesetzt wird, beruht nicht auf der bloßen Oxydationsbeschleunigung. Denn, wenn man dieselbe Oxydationsbeschleunigung durch Temperaturerhöhung herbeiführt, so wird, bei passender Wahl der Anfangstemperatur, die Entwicklung des Eies beschleunigt.

3. Loeb hat früher beobachtet, daß Zusatz von kleinen Mengen NaHO zu einer Mischung von $NaCl + KCl$ die Giftigkeit der Lösung erhöht, während Zusatz derselben Lösung zu $NaCl + CaCl_2$ (oder $NaCl + CaCl_2 + KCl$) die Giftigkeit der Lösung veringert. Dieser Unterschied beruht anscheinend nicht auf Verschiedenheiten der Oxydationsbeeinflussung der Eier in diesen Lösungen, da es sich zeigen läßt, daß die Oxydationsbeeinflussung der Eier von Arbacia in Woods Hole durch NaHO in allen drei Lösungen nahezu von der gleichen Größenordnung ist.

4. Die Oxydationsgeschwindigkeit der Eier von Arbacia in neutralen Lösungen von $NaCl + KCl$ ist nahezu von derselben Größenordnung wie in $NaCl + CaCl_2$ oder $NaCl + KCl + CaCl_2$. Der von Meyerhof für das Ei von Strongylocentrotus gezogene Schluß, daß die Gegenwart von Ca dessen Oxydationen auf das 12 bis 15fache ihres normalen Wertes herunterdrücke, läßt sich für die Eier von Arbacia nicht bestätigen.

5. Es wird gezeigt, daß bei gleicher (aber niedriger) Konzentration NH_4HO die Geschwindigkeit der Oxydation im befruchteten Ei von Arbacia ungefähr ebenso stark erhöht wie NaHO. Da Loeb schon früher gezeigt hat, daß bei der Entwicklung des Eies von Strongylocentrotus NaHO selbst durch eine so schwache Base wie Neutralrot ersetzt werden kann, so ist es zweifelhaft, ob es sich hier um Wirkung der freien HO-Ionen handelt. Es liegt näher an eine Wirkung der Basen vermittels Salzbildung zu denken.

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[Aus dem Department of Physiology and Pharmacology of the Rockefeller Institute, New York.]

Anaphylaxie und Atropin.

Erwiderung auf den Artikel von S. Mita.

Von **John Auer.**

(Eingegangen bei der Redaktion am 9. Dezember 1911.)

Vor einigen Jahren erschien eine Arbeit von Lewis und mir[1]) über den anaphylaktischen Tod beim Meerschweinchen, deren Ergebnisse ich hier kurz wiedergeben möchte, damit die Hauptpunkte dieser Erwiderung leichter verständlich werden. Lewis und ich zeigten zum ersten Mal: 1) daß der akute anaphylaktische Tod beim Meerschweinchen ein Erstickungstod ist; 2) daß diese Asphyxie durch eine Stenose der feineren Bronchien bedingt wird; 3) daß diese funktionelle Störung der Lungentätigkeit in einer augenfälligen, andauernden Blähung der Lungen ihren Ausdruck findet. Die Lunge bewahrt ihre Inspirationsstellung, selbst nachdem sie aus dem Thorax entfernt worden ist. Die Lunge ist bläulich-rosa, federleicht und zeigt auf der Schnittfläche nur geringfügige Spuren von Oedem; 4) daß die Ursache dieser außerordentlichen Lungenblähung in einer tetanischen Kontraktion der Muskulatur

1) Auer and Lewis, Journal of the American Medical Association, Vol. 53, 1909, p. 458; siehe auch Journal of Experimental Medicine, Vol. 12, 1910, p. 151.

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der kleineren Bronchien zu suchen ist[1]); 5) daß die Intoxikation peripher in den Lungen selbst angreift. Der Beweis für diesen Satz wurde dadurch erbracht, daß die typische Lungenblähung auch dann prompt eintrat, wenn die toxische Dosis nach vollständiger Zerstörung des Rückenmarks, der Medulla und der basalen Hirnteile, eingespritzt wurde[2]); 6) daß Atropin eine prophylaktische Wirkung bei der Meerschweinchenanaphylaxie entfaltet.

Diese Angaben sind nicht ungestützt geblieben, und ich möchte speziell auf die Arbeit von Biedl und Kraus[3]) verweisen. Anderson und Schultz[4]) untersuchten den Einfluß von Atropin auf die Meerschweinchenanaphylaxie, aber infolge ihrer Methoden vermochten sie nur 28 Proz. der Tiere zu retten. Karsner und Nutt[5]) untersuchten das quantitative Verhältnis zwischen Atropin und Multipla der tödlichen Dosis Pferdeserums.

In letzterer Zeit jedoch sind zwei der oben angeführten Sätze von Mita[6]) in Zweifel gezogen worden. Diese Zweifel sind aber, wie unten gezeigt werden wird, vollständig unbegründet.

Zuerst soll auf Mitas Bedenken gegen unsere Annahme eines peripheren Ursprungs der Anaphylaxie beim Meerschweinchen eingegangen werden. Mita ist augenscheinlich der Ansicht, daß das Atropinexperiment die einzige Stütze für unsere Folgerung sei, denn er erwähnt nur (p. 501), daß ich die präventive Wirkung des Atropins als Beweis für die „vermeintliche periphere Wirkung“ in der Anaphylaxie an-

1) Schultz und Jordan (Journal of Pharmacology and Experimental Therapeutics, Vol. 2, 1911, p. 375) haben kürzlich diese Muskelkontraktion mikroskopisch näher untersucht und verlegen sie hauptsächlich in die sekundären und tertiären Bronchien.

2) Die Beweise, mit graphischen Belegen, für die anderen Sätze sind ausführlich in der Arbeit im Journal of Exp. Medicine, l. c., angegeben.

3) Biedl und Kraus, Wiener klin. Wochenschr., Bd. 23, 1910. p. 387.

4) Anderson and Schultz, Proc. of Soc. for Exp. Biol. and Med., Vol. 7, 1909, p. 35.

5) Karsner and Nutt, Procedings of the Soc. for Exp. Biol. and Medicine, Vol. 8, 1911, p. 108; auch Journal of the Amer. Medical Assoc., Vol. 62, 1911, p. 1023.

6) Mita, Zeitschr. f. Immunitätsforschung, Orig., Bd. 11, 1911, p. 501.

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führe. Da nun Mita nur minimale Erfolge mit Atropin erhielt, so schätzt er diesen Beweis recht gering. Mita hat aber einen Abschnitt unserer Arbeit übersehen, in dem zweifellos die periphere Entstehung der Lungenstarre und -blähung bewiesen wird. Schon in unserer ersten Mitteilung schrieben Lewis und ich, daß die typische Lungenstarre und -blähung auch dann prompt einsetzt, wenn die toxische Dosis Pferdeserum erst nach gründlicher Zerstörung des gesamten Zentralnervensystems eingespritzt wird[1]). Durch dieses Experiment ist also die periphere Genesis der Anaphylaxie beim Meerschweinchen über allen Zweifel erhoben worden[2]).

Mita ist dieser Teil unserer Arbeit unbekannt geblieben, denn sonst hätte er unmöglich an der peripheren Entstehung der Lungenblähung zweifeln können.

1) Um dieses Experiment auszuführen, ist künstliche Atmung natürlich notwendig; auch muß man sich vergewissern, daß das Herz regelmäßig weiterschlägt. Um einer tödlichen Reflexhemmung des Herzens während der Zerstörung des Nervensystems vorzubeugen, sind die Vagi vorher durchzuschneiden.

2) In der Literatur über die experimentelle Untersuchung der Anaphylaxie ist mir kein Versuch bekannt, der klar und deutlich für eine primäre Wirkung auf das Zentralnervensystem spräche. Beim Meerschweinchen greift die toxische Dosis peripher in der Lunge an, und ich konnte den Nachweis führen, daß die enervierte Bronchialmuskulatur sich genau wie die normale in der Anaphylaxie verhält (Journal of Exp. Medicine, Vol. 12, 1910, p. 647). Biedl und Kraus (Wiener klin. Wochenschr., Bd. 22, 1909, p. 366) zeigten, daß die charakteristische Blutdrucksenkung beim Hunde durch eine Lähmung der vasokonstriktorischen Nervenendigungen im Splanchnicusgebiet bedingt wird. Ja, Eisenbrey (Procedings of the Soc. for Exp. Biology and Medicine, Vol. 7, 1910, p. 116) konnte sogar zeigen, daß die Biedl und Kraussche Blutdrucksenkung nicht eintritt, wenn die Reinjektionsdosis nur die Hirngefäße und nicht das Splanchnicusgebiet durchströmt; sie tritt jedoch prompt in Erscheinung, sobald die toxische Dosis in die Rumpfgefäße einfließt. Diese Versuche von Eisenbrey, mit Zirkulationsverkoppelung von zwei Hunden, beweisen strikt, daß die Blutdrucksenkung nicht zentralen Ursprunges ist. Und beim Kaninchen zeigte ich (Centralbl. f. Physiol., Bd. 24, 1911, No. 21; Journal of Exp. Med., Vol. 14, 1911, p. 476), daß der plötzliche anaphylaktische Tod durch eine Lähmung und starreähnlichen Zustand des Herzens hervorgerufen wird. Man sieht also, daß die Hauptsymptome in den drei verschiedenen Tierarten glatt durch periphere Veränderungen erklärt werden.

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Ich wende mich jetzt zu der Atropinfrage. Der vorläufigen Mitteilung von Lewis und mir über den therapeutischen Wert einer präventiven Atropineinspritzung folgte bald eine ausgedehntere Reihe von Versuchen, in der zahlenmäßig die Wirkung von einer vorausgehenden Atropininjektion auf die Dosis letalis minima des inaktivierten Pferdeserums ausfindig gemacht wird [1]). Von fünfundzwanzig (25) Atropin-Meerschweinchen blieben achtzehn (18) am Leben, während von vierundzwanzig (24) adäquaten Kontrolltieren achtzehn (18) prompt starben. Mit anderen Worten, 72 Proz. der Atropinreihe blieben **am Leben**, während 75 Proz. der Kontrolltiere **starben**. Dieses sehr günstige Ergebnis bemängelt Mita und schreibt (p. 501), sie seien nur scheinbar und dadurch bedingt, daß ich nicht mit der absoluten Dosis letalis gearbeitet hätte, da ja 25 Proz. der Kontrolltiere mit dem Leben davongekommen wären. Was Mita mit diesem Einwande meint, ist mir nicht verständlich, denn 75 Proz. der Kontrolltiere starben doch mit der Dosis letalis minima. Wenn nun in Betracht gezogen wird, daß drei Viertel der Kontrolltiere pünktlich starben, und daß praktisch drei Viertel (72 Proz.) ihrer Atropingenossen am Leben blieben, so erscheint der Schluß doch wirklich gerechtfertigt, daß Atropin eine ausgesprochene präventive Wirkung entfaltet, und es ist ohne Belang, ob nun die Dosis letalis eine Dosis minima oder eine Dosis absoluta war. Es ist deshalb augenscheinlich, daß die Erklärung Mitas nichts erklärt. Andere Erklärungsversuche macht Mita nicht.

Um Mitas Standpunkt verstehen zu können, ist es notwendig, seine Experimente etwas näher zu untersuchen. Mita arbeitete mit Meerschweinchen, die durch Pferdeserum, Taubenserum, Schweineserum und Rinderserum aktiv sensibilisiert wurden. Nach einigen Wochen gab er die absolute Dosis letalis stets intravenös. Bei den Atropintieren folgte die Antigeninjektion 15 Sekunden nach der intravenösen Einspritzung des Atropins. In der Pferdeserumreihe (p. 506) starben die Kontrolltiere in durchschnittlich 7 Minuten, die Atropintiere jedoch im Durchschnitt erst nach 39 Minuten. In

1) Auer, American Journ. of Physiol., Vol. 27, 1910, p. 439.

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den anderen Versuchsreihen ist aber der Atropineffekt praktisch null, obwohl in jeder ein Tier am Leben blieb. Bei oberflächlicher Betrachtung dieser Versuche scheint es demnach, als ob wirklich das Atropin nur eine geringfügige Wirkung ausübe und daß zwischen Mitas und meinen Versuchen ein grelles Mißverhältnis herrsche. Näheres Zusehen aber zeigt deutlich, wo der Fehler in Mitas Versuchen liegt: Mita begnügte sich in allen Anaphylaxieexperimenten mit der Einspritzung von zwei Milligramm Atropin, und diese Gabe war nicht groß genug, um die Tiere vom Tode zu retten. Mitas Uebersichtstabellen zeigen dies deutlich; in der Pferdeserumserie (p. 506) blieben die Atropintiere länger am Leben als die Kontrolltiere, in einem Falle sogar über 2 Stunden, während die Kontrolltiere nach 4—11 Minuten eingingen; und in jeder der Versuchsreihen mit Tauben-, Schweine- und Rinderserum blieb je ein Tier am Leben, und dieses Resultat darf wohl auf Rechnung des Atropins gesetzt werden, da ja Mita mit der Dosis letalis absoluta arbeitete. Seine eigenen Versuchsergebnisse hätten demnach Mita zu der Erkenntnis führen sollen, daß seine Atropingaben zu klein waren, um die funktionellen Störungen vollständig zu neutralisieren. Es muß eben bei jeder Versuchsreihe die Dosis efficiens des Atropins neu bestimmt werden, denn sie schwankt mit der Höhe der Sensibilisation der Tiere Praktisch bietet dies keinen Uebelstand, wenn man gleich von Anfang eine Dosis von 5—10 mg einspritzt, wie Biedl und Kraus es machten, oder injiziert, wenn nötig, mehr Atropin in die Jugularis, wie ich es in meinen Experimenten tat.

Weshalb Mita immer nur 2 mg Atropin injizierte, ist nicht recht klar. Er sagt zwar (p. 503), daß die Dosis in den einzelnen Versuchen jener von Auer und Lewis entspräche[1]), aber er vergißt, daß er nicht mit der minimalen Dosis letalis des Antigens arbeitete wie wir, sondern mit der absoluten tödlichen Dosis; schon deshalb hätte er auch die Atropingabe erhöhen sollen, denn es ist selbstverständlich, daß, wenn die Reinjektionsdosis vergrößert

1) Auer, American Journ. of Physiol., loc. cit. p. 449.

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wird, auch das Atropin eine entsprechende Zunahme erfahren muß, da ja die beiden als Antagonisten einander gegenüberstehen.

Uebrigens hätte Mita die Zahlenangabe nicht so wörtlich nehmen sollen, denn schon eine flüchtige Durchsicht meiner Versuchstabellen zeigt, daß oft mehr als 2 mg Atropin mit bestem Erfolg eingespritzt wurden, und zweitens waren Mita die guten Resultate bekannt, die Biedl und Kraus bei der präventiven Einverleibung von 5 mg erhielten. Durch dieses unzweckmäßige Festhalten an einer ungenügenden, starren Dosis Atropin hat Mita nur bewiesen, daß zwei Milligramm Atropin nicht genügten, um seine anaphylaktischen Meerschweinchen vom Tode zu retten. Mita jedoch ist anderer Ansicht; er schreibt wörtlich (p. 514): „Das Atropin hat bei der aktiven Anaphylaxie[1]) . . . nur einen minimalen Einfluß." Dieser Schluß ist jedoch viel zu weit gefaßt und wird nicht von Mitas Versuche bewiesen.

Zusammenfassung.

Mitas Annahme, daß das Atropinexperiment die Hauptstütze für den peripheren Ursprung der Anaphylaxie beim Meerschweinchen sei, entspricht nicht den Tatsachen.

Mitas Versuche sind so ausgeführt worden, daß sie seinen allgemeinen Schluß, Atropin übe nur einen minimalen Einfluß auf die Anaphylaxie aus, nicht erlauben.

Mitas Mißerfolg, seine Tiere durch Atropin zu retten, ist durch seine ungenügende Dosis dieses Mittels zwanglos zu erklären.

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1) Ich habe keine Veranlassung, auf Mitas Versuche mit primär giftigem Normalserum einzugehen, und möchte nur bemerken, daß die Symptomenkomplexe, welche unter anderem primär giftiges Serum und Friedbergers Anaphylatoxin auslösen, nicht mit der wirklichen Anaphylaxie verwechselt werden dürfen. Wie diese Verwechslung sicher vermieden werden kann, habe ich an anderer Stelle kurz beschrieben (Journ. of Experimental Medicine, Vol. 14, 1911, No. 5, p. 492—494).

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„Separatabdruck aus dem Zentralblatte für Physiologie, Band XXV, Nr. 22."

Die Bedeutung der Anpassung der Fische an den Untergrund für die Auffassung des Mechanismus des Sehens.

Von **Jacques Loeb** (Rockefeller Institute, New York).

(Der Redaktion zugegangen am 1. Jänner 1912.)

Über den Mechanismus der Gehirntätigkeit haben wir bis heute nicht einmal Vermutungen. Wir können nicht in das tätige Gehirn blicken und was an objektiven Manifestationen der Gehirntätigkeit der Beobachtung und Messung zugänglich ist, ist im allgemeinen so grundverschieden von der äußeren Reizursache, daß wir aus diesen Beobachtungen keine Schlüsse auf die Natur der Gehirnvorgänge ziehen können. Ich möchte nun in dieser Notiz auf ein biologisches Tatsachengebiet aufmerksam machen, welches vielleicht gestattet, einen Einblick in den Mechanismus zu gewinnen, welcher den Raumempfindungen zugrunde liegt.

Munk hatte behauptet, daß eine Projektion der Retina auf die von ihm als Sehsphäre bezeichnete Rinde des Hinterhauptlappens existiere und daß Exstirpation bestimmter Stellen dieser angeblichen Sehsphäre Erblindung bestimmter Partien der Netzhäute herbeiführe. Bei einer Wiederholung der Versuche Munks am Hunde war ich nicht in der Lage seine Angaben zu bestätigen. Henschen hat inzwischen auf Grund ausgezeichneter Beobachtungen am Menschen den Nachweis geliefert, daß eine solche Projektion doch existiert, daß sie aber in einem andern Teile des Gehirns liegt als Munk vermutete, nämlich in der Area striata. Minkowski konnte bei Versuchen am Hunde die Richtigkeit der Schlüsse von Henschen bestätigen. Diese Beobachtungen und Versuche weisen auf die Möglichkeit hin, daß beim Sehen ein Bild nicht nur auf der Retina, sondern auch auf der Großhirnrinde entsteht.

Diese Möglichkeit, daß dem Sehen die Entstehung eines Bildes im Gehirne zugrunde liegt, erhält eine Stütze durch ein Tatsachengebiet, das meines Wissens für diesen Zweck bisher nicht berücksichtigt worden ist.

Seit langer Zeit ist die Tatsache bekannt, daß viele Tiere, namentlich gewisse Fische, ihre Farbe und auch ihre Zeichnung dem Untergrund „anpassen". Man hat diese Tatsache eingehend für die Theorie der Erhaltung der Arten verwendet. Diese Tatsachen enthalten aber, wie mir scheint, auch den Nachweis, daß im Gehirn ein Bild der gesehenen Gegenstände entstehen muß. Pouchet hat vor vielen Jahren gezeigt, daß die „Anpassung" der Fische an den Untergrund aufhört, sobald man denselben die Augen entfernt, oder sobald die Entstehung der Retinabilder infolge der Trübung der brechenden Medien des Auges unmöglich wird. Dieser ausnahmslos bestätigte Befund beweist, daß diese Übereinstimmung der Zeichnung und Färbung der Haut mit dem Grunde durch das Retinabild vermittelt wird; daß mit anderen Worten die bisher als Farbenanpassung und

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Anpassung an den Untergrund bezeichnete Erscheinung nur eine Übertragung des Retinabildes auf die Haut ist.

Es ist ferner nachgewiesen, daß Zerstörung der Optikusfasern und der Optikusganglien im Gehirn ebenso wirkt wie Exstirpation der Augen; und endlich ist es erwiesen, daß das Durchschneiden der Sympathikusfasern, die zu den Pigmentzellen der Haut gehen, ebenfalls die Entstehung des Hautbildes des Untergrundes unmöglich macht. Wir kennen also den Weg, vermittels dessen das Retinabild auf die Haut der Fische übertragen wird. Eine Station ist die Endigung der Optikusfasern im Gehirn. Da wir nun ein objektives Bild des Objektes auf der Retina der Fische nachweisen können; da wir nachweisen können, daß das Bild auf der Haut der Fische eine Wiedergabe des Retinabildes, nicht aber des Objektes selbst ist; da ferner die Übertragung des Retinabildes auf die Haut durch die Sehnerven stattfindet, so muß das Bild bei der Übertragung auf die Haut die zentralen Stationen des Sehnerven passieren.

Ein Bild besteht aus einer Schar von Punkten verschiedener Lichtintensität, deren gegenseitige Anordnung bestimmt und für den Gegenstand charakteristisch ist. Sumner hat nun gezeigt, daß gewisse Fische imstande sind, ziemlich komplizierte Muster (z. B. ein Schachbrett), die den Boden des Aquariums bilden, auf ihrer Haut zu reproduzieren. Diese Reproduktion des Musters wird durch Nebenumstände etwas gestört, aber wenn diese Nebenumstände in Abzug gebracht werden, so bleibt die Tatsache bestehen, daß das Hautbild ein ziemlich getreues Bild des Musters des Untergrundes ist. Es besteht also eine bestimmte Anordnung verschiedener Lichtpunkte auf der Netzhaut der Fische und eine ähnliche Anordnung der Lichtpunkte auf der Haut des Fisches. Es liegt nahe, anzunehmen, daß jeder Punkt des Retinabildes ein Reizpunkt ist, welcher einen entsprechenden Bildpunkt durch Vermittlung einer Nervenfaser in den primären Optikusganglien hervorruft. Jeder Bildpunkt in den primären Optikusganglien kann wieder als Reizpunkt angesehen werden, der durch Vermittlung einer besonderen Nervenfaser eine einzelne Chromatophore der Haut oder eine kleine Gruppe derselben in einem bestimmten Sinne beeinflußt. Bei der Mosaiknatur der Retina kann man sich nicht gut vorstellen, daß eine Übertragung des Netzhautbildes auf die Haut anders erfolgen kann, als indem die relative Anordnung der einzelnen Punkte des Netzhautbildes in den Optikusfasern und in den Endganglien des Optikus bei der Übertragung erhalten bleibt. Es muß unter dieser Annahme im primären Optikusganglion eine relative Verteilung der Reizintensitäten stattfinden, welche der Verteilung der Bildpunkte auf der Retina entspricht und welche wieder als Bild bezeichnet werden kann.

Diese Erfahrungen an Fischen und die hier daran geknüpften Erwägungen legen den Gedanken nahe, daß das Sehen eine Form der Telephotographie ist für Farbe sowohl wie für das Muster. Ich hoffe, in einer späteren ausführlicheren Arbeit diese Ideen weiter zu entwickeln.

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80. P. A. Levene und F. B. La Forge: Über die Hefe-Nucleinsäure. V. Die Struktur der Pyrimidin-Nucleoside.

[Aus dem »Rockefeller Institute for Medical Research«, New York.]

(Eingegangen am 19. Februar 1912.)

In einer früheren Mitteilung haben Levene und Jacobs[1]) die Ansicht ausgesprochen, daß die organischen Komplexe der Hefenucleinsäure in zwei Klassen einzuteilen sind: die der Purinbasen und die der Pyrimidinbasen. Die ersteren besaßen alle Eigenschaften der Glykoside; über die Struktur der anderen konnte bisher eine Vermutung nicht ausgesprochen werden.

Die Pyrimidinkomplexe unterschieden sich von den anderen in Folgendem: 1) in großer Resistenz gegen die hydrolysierende Wirkung von verdünnten Mineralsäuren. Darin unterscheiden sich diese Körper nicht nur von den Purinnucleosiden, sondern auch von allen bekannten Glykosiden. Auf dieser Eigenschaft beruht die Darstellungsmethode der Substanzen. Wurde zur Hydrolyse konzentrierte Mineralsäure verwandt, so erhielt man die Pyrimidinbasen, während der Rest des Moleküles durch Zerstörung ganz verloren ging. 2) Bei der Behandlung mit Orcin geben die Pyrimidinkomplexe eine ganz unbedeutende Färbung. 3) Bei der Destillation mittels Salzsäure vom spezifischen Gewicht 1.06 ließ sich aus den Pyrimidinkomplexen nur eine unbedeutende Menge Furfurol abdestillieren, während einer Zeit, welche zur Bildung von Furfurol aus den Purinnucleosiden ausreichte, und 4) erwiesen sich die Pyrimidinsubstanzen widerstandsfähig gegen die Enzyme, welche die Purinnucleoside mit Leichtigkeit spalteten[2]).

Ein weiterer Widerspruch gegen die Annahme der glykosidartigen Natur der Pyrimidinkomplexe lag darin, daß aus dem Cytidin nur ein Tribenzoylderivat sich bildete, während aus einem Pentosid des Cytidins ein Tetraderivat entstehen sollte.

Da alle Bemühungen, das Cytidin oder das Uridin in ihre Komponenten zu spalten, versagten, wurde versucht, solche Derivate des stickstofffreien Teiles der Substanz zu gewinnen, welche die Aufklärung ihrer Natur möglich machten. Als erstes Orientierungsexperiment wurde der Versuch gemacht, die Substanzen einer lang dauernden Destillation mittelst Salzsäure vom spezifischen Gewicht 1.06 zu unterwerfen. Es zeigte sich dabei, daß die Entwicklung von Furfurol eine ganz träge und langdauernde war, so daß sie erst nach

[1]) B. 44, 1027 [1911].

[2]) Levene und Medigreceanu, Journal of Biological Chemistry 9, 389 [1911].

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36 Stunden beendigt war. Dabei entwickelte sich das Furfurol in einer Quantität, die auf ein äquimolekulares Verhältnis von Ribose und Base im Pyrimidin-Komplex deutete.

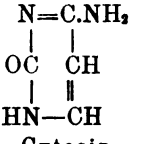
Auf diesen Befund hin wurde versucht, die Hydrolyse der Substanzen unter solchen Bedingungen auszuführen, welche die vollständige Zerstörung der Pentose verhinderten. Es sollte deswegen die Hydrolyse mittels Bromwasserstoffsäure in Gegenwart von Brom ausgeführt werden, in der Hoffnung, daß dabei die entstandene Pentosensäure vor weiterer Zersetzung geschützt sein würde. Das Verfahren wurde bereits von Neuberg mit Erfolg bei der Spaltung der Glucuronsäure-Verbindungen angewandt. In der Tat erhielten wir bei diesen Experimenten 5-Brom-uridin und *d*-Ribonsäure, welche in Form ihres Cadmiumsalzes und ihres Lactons identifiziert wurde.

Auf diese Weise wurde bewiesen, daß die Pyrimidinkomplexe aus Pyrimidin und Ribose zusammengesetzt sind. Die Elementaranalyse sprach für eine anhydridartige Verbindung. Vieles sprach aber auch dagegen, wie schon früher erwähnt wurde. Darum war es nötig, weitere Tatsachen zu finden, um die Aufklärung der Bindungsart der beiden Substanzen zu ermöglichen. Zunächst war es wichtig, darüber Klarheit zu verschaffen, ob die Doppelbindung in Stellung 4—5 noch im Komplexe vorhanden war. Behrend und seine Mitarbeiter haben bewiesen, daß bei der Einwirkung von Brom auf Pyrimidine die 4-Oxy-5-brom-dihydro-Derivate erhalten werden. Diese Substanzen haben die wichtige Eigenschaft, mit Phenylhydrazin charakteristische Derivate zu bilden und Fehlingsche Lösung beim Kochen zu reduzieren. Es erwies sich nun, daß Uridin bei der Behandlung mit Brom sich gerade wie Uracil verhielt. Wird eine wäßrige Lösung von Uridin oder Cytidin mit Brom bis zum Bestehenbleiben der gelben Farbe versetzt und der Überschuß von Brom durch Kochen entfernt, so reduziert die Lösung beim Kochen Fehlingsche Lösung; beim Erhitzen mit Phenylhydrazin bildet diese Lösung einen krystallinischen Niederschlag, der eine konstante Zusammensetzung und einen konstanten Schmelzpunkt besitzt. Beim Eindampfen des ursprünglichen Reaktionsproduktes gelangt man zum 5-Brom-uridin. Dadurch ist bewiesen, daß in dem Komplex die Doppelbindung noch intakt war.

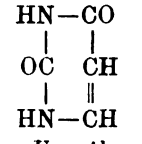
Auch durch das Verhalten gegen konzentrierte Salpetersäure wurde dieselbe Tatsache bewiesen. Auch hier zeigte sich die eigentümliche Widerstandskraft dieser Komplexe gegen oxydierende und hydrolysierende Mittel. Wurde das Uridin mit konzentrierter Salpetersäure auf dem Wasserbade zu einem Syrup eingedampft, so er-

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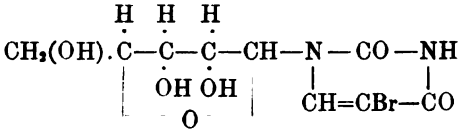
hielt man dabei eine Verbindung, die nach ihrer Zusammensetzung eine anhydridartige Verbindung von 2 Molekülen einer Nitro-uridin-carbonsäure war. Diese Substanz ließ sich leicht in ihre Äthyl- oder Butylester überführen. Durch Hydrolyse der Substanz ließ sich Nitro-uracil darstellen, welches identisch war mit der Substanz, die Behrend bei der Behandlung von Uracil mit Salpetersäure erhielt. Ein Versuch, das Nitro-uridin in Amino-uridin durch Reduktion mit Salzsäure und Zinn überzuführen, gelang nicht, da die Substanz sich dabei unter Bildung von Isobarbitursäure spaltete. Aus diesem Versuche konnte man schließen, daß die Doppelbindung in Uracil intakt war und auch, daß die Bindung der beiden Komponenten verhältnismäßig widerstandsfähig ist. Es blieb jedoch unklar, auf welche Weise und in welcher Stellung die Bindung von Ribose und Uracil statthat. Wenn man aber die Strukturformeln von Uracil und von Cytosin in Betracht zieht und auch die Tatsache berücksichtigt, daß Cytidin leicht in Uridin und weiter, daß Uridin leicht in 5-Bromuridin und in 5-Nitrouridincarbonsäure übergeführt werden kann, so gelangt man zu der Überzeugung, daß die Bindung nur in den Stellungen 3 oder 4 eingetreten sein kann:

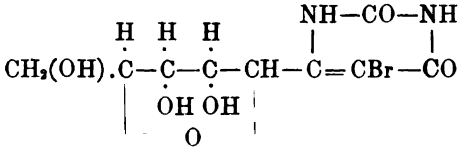


Cytosin



Uracil





Um Klarheit in dieser Frage zu bekommen, wurde versucht, Alkylderivate des Uridins oder des Cytidins darzustellen. Alle Bemühungen aber, aus den Alkali-, Blei- oder Silbersalzen dieser Substanzen Methyl- oder Äthylderivate darzustellen, mißlangen. Ein Teil der Substanz wurde unverändert wiedergefunden, der andere ging unter Zersetzung verloren. Wir konnten deswegen über diesen Punkt zu keiner sicheren Ansicht gelangen.

Nun war es aber möglich, das Uridin und das Cytidin leicht zu Dihydroverbindungen zu reduzieren, und dabei kam die merkwürdige Tatsache zum Vorschein, daß das Dihyro-uridin und das Dihydro-cytidin ihre Widerstandsfähigkeit gegen die hydroly-

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sierende Wirkung von verdünnten Mineralsäuren verloren hatten. Diese Substanzen ließen sich leicht in *d*-Ribose und in die Dihydro-Basen zerlegen. Die Ribose konnte alsdann in das Phenylosazon übergeführt und als solches identifiziert werden. Man kann also mit einem gewissen Maße von Wahrscheinlichkeit annehmen, daß die Widerstandskraft der ursprünglichen Pyrimidinkomplexe darauf beruht, daß die glycosidartige Bindung zwischen der Ribose und der Base in Stellung 5 sich befindet, und daß es die Nachbarschaft der Doppelbindung ist, welch der Substanz ihre Widerstandskraft verleiht. Unzweideutige Beweise für diese Ansicht fehlen allerdings noch. Es ist auch erwähnenswert, daß die Dihydroverbindungen ähnlich den Purinnucleosiden linksdrehend sind, während die ursprünglichen Pyrimidinkomplexe nach rechts drehen.

Es soll hier auch angeführt werden, daß zur Ausführung dieser Arbeit große Quantitäten von Uridin nötig waren, und daß das alte Verfahren zur Darstellung der erforderlichen Mengen viel zu umständlich war. Es gelang uns, die Substanz mit großer Leichtigkeit zu erhalten, indem wir den Zucker in sein Glukosid überführten, und dadurch seine Fällbarkeit durch Blei und Baryt oder durch Blei und Ammoniak verhinderten[1]), während die Basen ihre Fällbarkeit diesen Reagenzien gegenüber bewahrten. Auf diese Weise gelangten wir auch zur Trennung des Uridins von der bei der Spaltung von Nucleinsäure sich bildenden Ribose, die große Schwierigkeiten bei der Isolierung des Uridins und des Cytidins bereitete.

Auch die Isolierung des Cytidins wurde durch die Beobachtung erleichtert, daß das Nitrat wegen seiner schweren Löslichkeit in Wasser sich gut zur Isolierung der Substanz eignet.

Auch gelang es uns jetzt, das freie Cytidin krystallisiert zu erhalten und seine Eigenschaften festzustellen. Wegen der großen Löslichkeit der Substanz in Wasser wurde sie bis jetzt nur in Form ihrer Salze und des Tribenzoylderivats erhalten.

Experimenteller Teil.

Isolierung des Cytidins als Nitrat.

200 g Hefenucleinsäure werden in der üblichen Weise hydrolysiert und auf Guanosin und Adenosin verarbeitet. Das Filtrat

[1]) Die Überführung des Zuckers in sein Glucosid wurde ausgeführt nach dem Verfahren, das Jacobs in diesem Laboratorium als sehr günstig für die allgemeine Darstellung von Glucosiden erprobt hat. Es besteht darin, daß der Zucker in Alkohol gelöst, die Lösung, mit Salzsäure gesättigt und dann der Überschuß der Säure mit Bleicarbonat entfernt wird.

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vom Adenosinpikrat wird von Pikrinsäure durch Ausschütteln mit Äther und von Schwefelsäure durch Bariumcarbonat befreit und im Vakuum auf ca. 75 ccm eingedampft. Es werden dann ca. 10 ccm Salpetersäure und darauf ca. 75 ccm Alkohol zugegeben. In wenigen Minuten ist die Lösung zu einem dicken Brei von Krystallen erstarrt. Nach dem Abfiltrieren, Waschen mit 50-prozentigem Alkohol und Trocknen wiegt das fast ganz reine Produkt 15—17 g. Für die Analyse wurde es einmal aus Wasser umkrystallisiert. Schmp. 197° (unkorr.).

Die Analyse des Nitrats ergab die folgenden Zahlen:

0.1086 g Sbst.: 17.5 ccm N (21.5°, 762 mm)[1]).

$C_9H_{13}N_3O_5$, HNO_3. Ber. N 18.34. Gef. N 18.36.

Cytidin. Die freie Base wurde nur unter folgenden Bedingungen krystallisiert erhalten. Ein Teil Cytidinsulfat, das dreimal aus Wasser umkrystallisiert worden war, wurde in wenig Wasser gelöst und mit der genau berechneten Menge Bariumhydroxyd, das ebenfalls durch wiederholtes Umkrystallisieren gereinigt war, versetzt. Die Lösung wurde durch ein gehärtetes Filter filtriert und in einer Glasschale bei gewöhnlicher Temperatur im Vakuum über Calciumchlorid zur Trockne verdunstet. Die farblose, glasige Masse wurde dann in heißem, absolutem Alkohol gelöst; aus dieser Lösung krystallisierte die freie Base beim Reiben mit einem Glasstab. Die Krystalle, die nunmehr in absolutem Alkohol ziemlich schwer löslich waren, wurden aus 90-prozentigem Alkohol umgelöst. Der Körper krystallisiert daraus in langen Nadeln, die bei 220° sintern und bei 230° sich zersetzen.

0.1366 g Sbst.: 21.2 ccm N (21°, 761 mm).

$C_9H_{13}N_3O_5$. Ber. N 17.28. Gef. N 17.57.

0.3501 g Sbst. (in 3.5 ccm Wasser, Gesamtgewicht der Lösung 3.8331 g) drehten im 1-dm-Rohr bei 21° und Natriumlicht 2.71° (± 0.03°) nach rechts. Mithin $[\alpha]_D^{21} = +29.63°$ (± 0.3°).

Furfurol-Destillation des Cytidinsulfats. 0.6150 g Substanz wurden mit Salzsäure vom spez. Gewicht 1.06 destilliert. Die Salzsäure ließ man von Zeit zu Zeit aus einem Tropftrichter zufließen. Das Destillat wurde alle zwei Stunden mit essigsaurem Anilin auf Anwesenheit von Furfurol geprüft. Die Destillation wurde über Nacht unterbrochen und am folgenden Tage fortgesetzt. Nach 36 Stunden gab das Destillat keine Rotfärbung mit essigsaurem Anilin mehr, worauf die Destillation beendet wurde. Das Furfurol wurde als

[1]) Der Stickstoff wurde hier, wie bei den nachfolgenden Bestimmungen, über Wasser gemessen.

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Phloroglucid niedergeschlagen und gewogen. Seine Menge betrug 0.2642 g. Da die Ausbeute, welche Ribose an Furfurol liefert, noch nicht bekannt ist, wurde die Substanz auf Arabinose berechnet; sie entsprach 0.3037 g, während die Theorie für Ribose 0.3146 verlangt.

Gewinnung des Uridins.

Für die Darstellung dieses Körpers kann man entweder ebenfalls von dem Filtrat vom Adenosinpikrat, wie beim Cystidin ausgehen oder wenn man Zeit und Reagenzien sparen will, unter Opferung des Adenosins, das zum Sirup eingedampfte Filtrat von Guanosin verwenden.

Das Filtrat von Guanosin, das man bei der Hydrolyse aus 200 g Hefenucleinsäure erhält, wird in einer Schale auf dem Dampfbad auf ca. 75 ccm eingedampft und der dunkle Sirup mit Hilfe von ca. 50 ccm heißem Wasser in einen 2-l-Fraktionierkolben gespült. Es werden darauf $1^1/_2$ l absoluten Alkohols zugegeben und die Lösung mit Chlorwasserstoffgas gesättigt.

Während des Einleitens läßt man die Temperatur ruhig steigen, bis der Alkohol beinahe zu sieden anfängt, kühlt dann ab und fährt mit dem Einleiten fort, bis die Lösung gesättigt ist.

Hierdurch wird erstens Spaltung der Puringlucoside und teilweise Ausscheidung der Purine als Chlorhydrate, sowie eine teilweise Ausscheidung des Cytidins als salzsaures Salz erreicht; zweitens verwandelt sich auch der freie Zucker in das Äthylglucosid. Ohne von dem ausgeschiedenen Material abzufiltrieren, wird etwa $^1/_3$ der alkoholischen Salzsäure im Vakuum abdestilliert und dann nach Zugabe des gleichen Volumens Alkohol auf der Nutsche filtriert und mit Alkohol gut gewaschen. Das Filtrat hat jetzt ein Volumen von $3^1/_2$ l. Es wird nun unter Rühren Bleizucker, der in 95-proz. Alkohol heiß gelöst ist, zugegeben, bis die Lösung nicht mehr sauer auf Congo reagiert, und dann noch ca. 100 g im Überschuß. Es ist ca. 1 kg Bleizucker erforderlich, das man in $1^1/_2$ l Alkohol heiß löst. Der Bleizucker bewirkt Ausscheidung der Phosphorsäure sowie der Hauptmenge der Salzsäure. Nach dem Filtrieren und Waschen des Niederschlages mit Alkohol wird das Filtrat auf ca. 1 l im Vakuum eingeengt, auf 3 l mit Wasser verdünnt und so viel aufgeschlämmtes Silberoxyd zugegeben, bis die Lösung eine positive Reaktion auf Silber zeigt. Nach einigem Stehen wird von dem schmutzigen Niederschlag abfiltriert und das Filtrat von Blei und Silber durch Schwefelwasserstoff befreit. Das Silberoxyd bewirkt die Entfernung der noch vorhandenen Purine und der Salzsäure und wahrscheinlich auch noch anderer Verunreinigungen. Noch bevor man von den Sulfiden abfiltriert, gibt man so viel Bleizuckerlösung zu, bis der Überschuß an Schwefelwasserstoff abgesättigt ist. Nach dem Filtrieren und Waschen wird mit 350 ccm einer bei Zimmertemperatur gesättigten (ca. 40-proz.) Lösung von Bleizucker versetzt und die Lösung, die jetzt ein Volumen von ca. 6 l hat, mit einer heißen, konzentrierten Lösung von Bariumhydroxyd versetzt, bis die Fällung vollständig ist. (Der Niederschlag, der sich bei der

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ersten Zugabe von Barythydrat bildet, löst sich wieder auf.) Ein Überschuß von Barythydrat ist streng zu vermeiden. Der sehr voluminöse weiße Niederschlag wird auf der Nutsche abfiltriert, gut gewaschen und in einer starken Flasche mit verdünnter Schwefelsäure in der üblichen Weise zerlegt. Ohne zu filtrieren, wird der Überschuß der Schwefelsäure durch Zugabe von wenig Bleicarbonat neutralisiert und dann nach dem Filtrieren der Überschuß von Blei durch Schwefelwasserstoff entfernt und die Lösung im Vakuum zum dicken Sirup konzentriert. Dieser wird in ca. 150 ccm Alkohol warm aufgenommen und ohne weiteres in einer Schale auf dem Wasserbade auf ca. 60 ccm eingeengt. Manchmal fängt schon während des Eindampfens, jedenfalls beim Reiben mit einem Glasstab, die Krystallisation des Uridins an, und nach wenigen Minuten ist der Inhalt der Schale zu einem Kuchen erstarrt. Nach einigem Stehen im Eisschrank wird die Krystallmasse mit wenig Alkohol angerieben und abfiltriert.

Ausbeute bis 20 g, normalerweise ca. 16 g.

Das so gewonnene Produkt ist fast ganz rein. Zur völligen Reinigung wird es in ca. 8 Teilen 90-proz. Alkohol gelöst, mit wenig Tierkohle versetzt und heiß filtriert. Aus der auf die Hälfte eingedampften Lösung krystallisiert das Uridin in rein weißen, großen Krystallen. Ausbeute an chemisch reiner Substanz (Schmp. 165°) 14—18 g. Hat man das Filtrat vom Adenosin-pikrat verwendet, so beträgt die Ausbeute nur ca. 10 g.

0.1501 g Sbst.: 0.2444 g CO_2, 0.0688 g H_2O.

$C_9H_{12}O_6N_2$. Ber. C 44.27, H 4.98.
Gef. » 44.35, » 5.08.

Aus dem Filtrat vom rohen Uridin kann man noch 2—3 g Cytidin als Nitrat gewinnen.

Molekulargewichtsbestimmung. 0.3628 g Sbst. in 25 ccm Wasser bewirkten eine Erniedrigung des Gefrierpunktes um 0.11°.

Ber. M 244. Gef. M 250.

Hydrolyse mittels Brom und Bromwasserstoffsäure.

15 g Cytidinnitrat wurden mit 250 ccm 10-prozentigem Bromwasserstoff und 20 g Brom 7 Stunden am Rückflußkühler gekocht und die Lösung dann in Vakuum eingedampft. Gegen Ende der Destillation findet eine Entwicklung von Brom statt, sowie Ausscheidung eines krystallisierten Körpers. Der Inhalt des Kolbens wird mit ca. 30 ccm Wasser aufgenommen und der Krystallisation überlassen. Die Menge des schwer löslichen Körpers betrug 2.5 g. Er wurde aus Wasser umkrystallisiert. Schmp. 293° (unkorr.). Er ist der Analyse und den Eigenschaften nach das 5-Brom-uracil[1]).

[1]) Am. 29, 486.

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0.1568 g Sbst.: 0.1588 g AgBr. — 0.1514 g Sbst.: 0.0240 g H_2O, 0.1406 g CO_2. — 0.1220 g Sbst.: 15.6 ccm N (27°, 765 mm).

$C_4H_3N_2O_2Br$. Ber. C 25.13, H 1.57, N 14.65, Br 41.88.
Gef. » 25.30, » 1.76, » 14.20, » 41.74.

Das Filtrat von Bromuracil wurde etwas verdünnt, mit einem Überschuß von reinem Silberoxyd behandelt, nach einigem Stehen filtriert und das Filtrat durch Schwefelwasserstoff von Silber befreit. Nachdem man den Schwefelwasserstoff durch Kochen vertrieben hatte, wurde in der üblichen Weise mit reiner Bleizuckerlösung und Barythydrat gefällt und der Bleiniederschlag mit verdünnter Schwefelsäure zerlegt. Der Überschuß der Schwefelsäure wurde mit wenig Bleicarbonat entfernt und in das Filtrat Schwefelwasserstoff eingeleitet. Nach dem Eindampfen auf ca. 50 ccm wurde alsdann so lange mit Cadmiumcarbonat gekocht, bis die Lösung nicht mehr sauer reagierte, nach dem Filtrieren wurde sodann auf dem Wasserbade auf ca. 8 ccm eingedampft. Nach dem Abkühlen wurde mit einem Kryställchen von ribonsaurem Cadmium geimpft und stehen gelassen. Nach einigen Tagen ist der Inhalt der Schale zu einem festen Kuchen erstarrt. Die rohe Substanz wurde mit wenig 50-prozentigem Alkohol angerieben und auf der Nutsche filtriert. Ihre Menge betrug 1.55 g.

Zur Analyse wurde aus ca. 2 ccm Wasser umkrystallisiert.

0.2132 g Sbst.: 0.1008 g $CdSO_4$.

$(C_5H_9O_6)_2Cd$. Ber. Cd 25.34. Gef. Cd 25.41.

0.1545 g Substanz in 1.54 ccm $^n/_1$-H_2SO_4, Gesamtgewicht der Lösung 1.72 g, drehten im 0.5-dm-Rohr 0.25° nach rechts [1]). Mithin $[\alpha]_{20}^D = +8.42°$.

Das Lacton der *d*-Ribonsäure wurde durch Zerlegung des Cadmiumsalzes durch Schwefelwasserstoff erhalten; es zeigte, aus Essigester umkrystallisiert, den Schmp. 72—78° [2]).

Phenylhydrazid. Dieses Derivat wurde genau nach Fischer und Piloty [3]) dargestellt. Aus 50-prozentigem Alkohol umkrystallisiert, zeigte es den unscharfen Schmp. 162° und zersetzte sich bei ca. 180° unter Gasentwicklung.

0.1560 g Sbst.: 15.6 ccm N (25°, 764 mm).

$C_{11}H_{16}N_2O_5$. Ber. N 10.94. Gef. N 11.27.

Bromierung des Uridins.

2 g Uridin werden so lange mit Bromwasser versetzt, bis die rote Farbe eben nicht augenblicklich verschwindet; dann wird die Lö-

[1]) Die Lösung dreht zunächst nach links und kommt erst nach einigem Stehen zu einer konstanten Rechtsdrehung.

[2]) B. 24, 4214 [1891]. [3]) Ibid.

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sung zum Sirup im Vakuum eingedampft. Dieser wird in absolutem Alkohol aufgenommen und in einer Schale auf dem Wasserbad unter Rühren mit einem Glasstab eingedampft, bis der Körper krystallinisch erstarrt. Nach dem Anreiben mit absolutem Alkohol und Trocknen wog das Produkt 2 g. Aus 5 Tln. 95-proz. Alkohol umgelöst, zersetzt sich der Körper bei 181—184° (unkorr.). Die gleiche Verbindung entsteht, wenn man eine konzentrierte Lösung von Uridin mit einem Überschuß von Brom behandelt und nach längerem Stehen mit Alkohol kocht.

Das Brom-uridin zeigt fast alle Eigenschaften des Uridins. Es reduziert Fehlingsche Lösung erst nach Behandlung mit Brom, auch gibt es eine gleich schwache Orcinreaktion. Das Brom läßt sich nur unter Zersetzung der Verbindung durch längeres Kochen mit starker Lauge abspalten.

0.1288 g Sbst.: 0.1568 g CO_2, 0.0420 g H_2O. — 0.1685 g Sbst.: 0.0976 g AgBr. — 0.1541 g Sbst.: 11.4 ccm N (20°, 765 mm).

$C_9H_{11}N_2BrO_6$. Ber. C 33.43, H 3.41, Br 24.76, N 8.66.
Gef. » 33.20, » 3.62, » 24.67, » 8.48.

0.6028 g Substanz in 6 ccm Wasser, Gesamtgewicht der Lösung 6.6054 g, drehten bei 21° und Natriumlicht −1.40° (± 0.02°). Mithin

$$[\alpha]_D^{21} = -15.38° \ (\pm 0.02°).$$

Oxy-uridin.

Als Zwischenprodukt bei der Darstellung des Brom-uridins entsteht ohne Zweifel das dem 4-Oxy-5-brom-dihydro-uracil analoge Derivat des Uridins, denn beim Kochen einer Lösung von Uridin, die man, wie beim Bromuracil, mit Brom behandelt hat, mit Bleioxyd wird das Brom quantitativ abgespalten.

2 g Uridin werden mit Bromwasser behandelt, bis die Farbe eben bestehen bleibt, dann wird die Lösung 1 Std. mit Bleioxyd gekocht. Die Flüssigkeit wurde, ohne zu filtrieren, auf 20 ccm eingedampft, nach dem Abkühlen mit ca. 2 Volumen Alkohol versetzt und nach einigem Stehen filtriert. Das Blei wurde durch Schwefelwasserstoff entfernt und die Lösung, die noch sauer auf Congo reagierte, zum Sirup eingedampft und im Exsiccator stehen gelassen. Es krystallisierte langsam ein Körper aus, der nach 2—3 Tagen von der anhaftenden Mutterlauge durch Streichen auf Ton befreit wurde. Die Krystalle wurden mit Alkohol angerührt und nach dem Filtrieren aus demselben Lösungsmittel umkrystallisiert. Schmp. 222—223° (unkorr.). Die Verbindung ist der Analyse nach Oxy-uridin.

0.1205 g Sbst.: 0.1822 g CO_2, 0.0533 g H_2O.

$C_9H_{12}N_2O_7$. Ber. C 41.53. H 4.61.
Gef. » 41.23, » 4.91.

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Phenylhydrazid. 1 g Uridin wurde bis zur bleibenden roten Farbe mit Bromwasser behandelt und nach dem Wegkochen des kleinen Überschusses des Broms mit 4 g Natriumacetat und 7 g Phenylhydrazin in Essigsäure versetzt. Beim Erhitzen auf dem Wasserbade fing bald die Ausscheidung der in ziegelroten Blättchen krystallisierenden Phenylhydrazin-Verbindung an. Nach 1½-stündigem Erhitzen wurde noch ca. 12 Stdn. bei gewöhnlicher Temperatur stehen gelassen und dann, nach dem Abfiltrieren, mit 50 ccm Alkohol aufgekocht. Es findet zunächst völlige Auflösung statt und gleich darauf Auskrystallisieren des Körpers in langen, citronengelben Nadeln, die nunmehr in Alkohol sehr schwer löslich sind. Der Körper schmolz bei 209° (unkorr.). Ausbeute 0 5 g.

Ihm kommt die empirische Formel $C_{15}H_{30}N_4O_5$ zu.

0.1215 g Sbst.: 0.2387 g CO_2, 0.0640 g H_2O. — 0.1319 g Sbst.: 0.2579 g CO_2, 0.0716 g H_2O. — 0.1232 g Sbst.: 17.7 ccm N (21°, 770 mm).

Ber. C 53.60, H 5.95, N 16.66.
Gef. » 53.61, 53.47, » 5.88, 5.96, » 16.49.

Da die Verbindung für die Aufklärung der Konstitution des Uridins ohne Belang erschien, wurde sie nich weiter untersucht.

Oxydation des Uridins mittels Salpetersäure.

5 g Uridin wurden in einer Glasschale mit 20 ccm Salpetersäure (1 Tl. konzentrierte Salpetersäure + 1 Tl. Wasser) auf dem Wasserbade zum Sirup konzentriert. Beim Reiben erstarrt dieser sofort zu einem harten Kuchen. Er wurde nach dem Abkühlen mit 75-prozentigem Alkohol angerieben und filtriert. Nach dem Umkrystallisieren aus 3—4 Tln. Wasser zeigt die Verbindung das Aussehen kurzer, dicker Prismen, die sich oberhalb 200°, ohne vorher zu schmelzen, zersetzen.

Die Analyse der Säure sowie die ihrer Ester zeigt, daß zwei Moleküle des Oxydationsproduktes anhydridartig zusammengetreten sind.

0.1388 g Sbst. neutralisierten 8.7 ccm $^n/_{10}$-Alkali (Phenolphthalein), (berechnet 9.3 ccm.) — 0.1594 g Sbst. verloren bei 100° im Vakuum 0.0097 g H_2O. — 0.1496 g wasserfr. Sbst.: 0.1994 g CO_2, 0.0418 g H_2O. — 0.1531 g wasserfr. Sbst.: 0.2039 g CO_2, 0.0420 g H_2O. — 0.1460 g wasserfr. Sbst.: 18.0 ccm N (22°, 768 mm).

$C_{18}H_{16}N_6O_{17} + 2H_2O$. Ber. H_2O 5.76. Gef. H_2O 6.08.
$C_{18}H_{16}N_6O_{17}$. Ber. C 36.49, H 2.72, N 14.19.
Gef. » 36.34, 36.30, » 3.10, 3.05, » 14.10.

Ein Molekül Säure neutralisiert 4 Moleküle Alkali, wie durch Titration und Darstellung des Silbersalzes festgestellt wurde.

Das Silbersalz der Nitro-uridin-carbonsäure entsteht bei Zugabe von Silbernitratlösung zu der Lösung des Alkalisalzes der Säure. Es ist amorph und wurde deshalb nur gewaschen und bei 100° im Vakuum getrocknet.

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0.2484 g Sbst.: 0.1041 g Ag.

$C_{18}H_{12}N_6O_{17}Ag_4$. Ber. Ag 42.3. Gef. Ag 41.9.

Äthylester und *n*-Butylester der Nitro-uridin-carbonsäure. Ein Teil Uridin wird mit Salpetersäure auf dem Wasserbade eingedampft, die entstandene Nitrosäure fein gepulvert und mit 10 Tln. äthylalkoholischer Salzsäure 15 Minuten über freier Flamme gekocht. Es findet Auflösung der Säure statt, und bald darauf krystallisiert schon in der Hitze der Ester aus, indem die Flüssigkeit sich breiartig mit feinen Nadeln erfüllt. Nach einigem Stehen im Eisschrank werden die Krystalle abfiltriert und mit Alkohol gewaschen. Die Ausbeute ist der Menge des angewandten Uridins gleich. Für die Analyse wurde einmal aus Alkohol umgelöst. Wie die Säure selbst, besitzt der Ester keinen Schmelzpunkt, sondern zersetzt sich oberhalb 200°.

0.1290 g Sbst.: 0.1925 g CO_2, 0.0484 g H_2O. — 0.1979 g Sbst.: 22.0 ccm N (21°, 765 mm).

$C_{22}H_{24}N_6O_{17}$. Ber. C 40.99, H 3.72, N 13.04.
Gef. » 40.73, » 4.17, » 12.63.

Der *n*-Butylester wird auf ganz analoge Weisa wie der Äthylester dargestellt. Aus Alkohol umkrystallisiert, zeigte er den Schmp. 190—192°, nachdem er von 185° an zu sintern begonnen hatte.

0.1230 g Sbst.: 0.2089 g CO_2, 0.0560 g H_2O.

$C_{26}H_{32}N_6O_{17}$. Ber. C 44.57, H 4.57.
Gef. » 44.45, » 4.86.

5-Nitro-uracil aus Nitro-uridin-carbonsäure.

2 g Säure wurden 3 Stunden im Rohr mit 20 ccm 20-prozentiger Schwefelsäure auf 130—135° erhitzt. Der Inhalt des Rohres hatte sich dabei unter Ausscheidung von Zersetzungsprodukten dunkel gefärbt. Er wurde verdünnt, mit Tierkohle ausgekocht und nach dem Filtrieren mit Quecksilbersulfat-Lösung gefällt. Der entstandene flockige Niederschlag wurde gewaschen und mit Schwefelwasserstoff zerlegt. Durch Eindampfen auf dem Wasserbade wurde unreines Nitro-uracil erhalten. Nach zweimaligem Umkrystallisieren aus Wasser wurde es getrocknet und analysiert. Ausbeute nur 0.2 g. Die Verbindung stimmte in ihren Eigenschaften mit dem von Behrend[1]) dargestellten Präparat vollständig überein.

0.1201 g Sbst.: 26.9 ccm N (19°, 770 mm).

$C_4H_3N_3O_4$. Ber. N 26.75. Gef. N 26.02.

Zur weiteren Charakterisierung wurde die Verbindung mit Guanidin dargestellt, die man durch Zusammenbringen der wäßrigen Lösungen von Nitro-uracil mit Guanidin-carbonat erhält. Auch diese Verbindung besaß ganz die von Behrend angegebenen Eigenschaften[2]).

[1]) A. **240**, 5. [2]) A. **240**, 18.

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0.0990 g Sbst.: 30.5 ccm N (20°, 774 mm).

$C_5H_8N_6O_4$. Ber. N 35.90. Gef. N 35.73.

Isobarbitursäure[1]). 3 g Nitro-uridin-carbonsäure wurden mit 40 ccm 20-proz. Chlorwasserstoffsäure und 12 g Zinn versetzt. Nachdem die Reaktion sich gemäßigt hatte, wurde 1 Stunde gekocht und, nach Zusatz von ca. 100 ccm Wasser, durch Schwefelwasserstoff entzinnt. Das Filtrat vom Zinnsulfid lieferte beim Eindampfen auf ein kleines Volumen 1.1 g Isobarbitursäure.

0.1471 g Sbst.: 27.1 ccm N (18°, 772 mm).

$C_4H_4N_2O_3$. Ber. N 21.87. Gef. N 21.46.

Es gelang nicht, das zweite Spaltungsprodukt zu fassen.

Reduktion des Uridins zu Dihydro-uridin.

4 g Uridin wurden im Apparat von Paal unter Zugabe von 0.2 g kolloidalem Palladium reduziert. Die Reduktion geht sehr langsam vor sich und ist erst nach 30—35 Stunden beendet. Die Reaktionsgeschwindigkeit scheint von der Menge des Palladiums abhängig zu sein. Nachdem das Palladium durch Zusatz von einigen Tropfen Essigsäure gefällt war, wurde davon abfiltriert und die Lösung auf dem Wasserbade zum Sirup konzentriert. Dieser wurde in absolutem Alkohol heiß gelöst und von einer geringen Menge, aus dem Palladiumpräparat herstammenden Eiweiß abfiltriert. Das Reduktionsprodukt hinterbleibt nach dem Verdunsten des Alkohols als farbloser Sirup, der nicht zum Krystallisieren gebracht werden konnte.

Ein quantitativer Versuch ergab folgendes Resultat:

0.5595 g Uridin verbrauchten 57.2 ccm H_2 (20°, 761 mm); ber. für H_2 57.5 ccm.

Eine wäßrige Lösung des Dihydro-uridins reduziert Fehlingsche Lösung gar nicht, ebenso wenig nach Behandlung mit Bromwasser. Dieses wird auch nicht entfärbt.

Sie gibt eine sehr starke Orcinreaktion, ähnlich wie die Puringlucoside, und nach der Hydrolyse kräftige Reduktion der Fehlingschen Lösung.

Eine wäßrige Lösung, deren Gehalt an Dihydrouridin aus dem Stickstoffgehalt zu 0.8056 g bestimmt war, in 20 ccm Wasser, Gesamtgewicht der Lösung 21 g, drehten in 2-dm-Rohr bei Natriumlicht 3.00° nach rechts. Mithin $[\alpha]_D = +39.1°$.

4 ccm der obigen Lösung wurden für eine Kjeldahl-Stickstoffbestimmung benutzt. Sie erforderte zur Neutralisation 13.0 ccm $^n/_{10}$-Schwefelsäure.

[1]) A. **229**, 39.

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Hydrolyse des Dihydro-uridins mittels verdünnter Schwefelsäure.

Eine Lösung von Dihydrouridin, das bei der Reduktion von 4 g Uridin entstanden war, wurde 1½ Stunden lang in 100 ccm 3-prozentiger Schwefelsäure am Rückflußkühler gekocht. Die Schwefelsäure wurde dann mit Bariumcarbonat gefällt und die Lösung in Vakuum auf 20 ccm eingedampft. Eine geringe Menge Barium wurde durch einige Tropfen verdünnter Schwefelsäure gefällt, und dann kochend heiß abfiltriert. Beim Abkühlen in Eiswasser, krystallisierten 1.4 g Dihydro-uracil aus, das zur Analyse einmal aus Wasser umkrystallisiert wurde. Es zeigte den Schmp. 274°, wie für Dihydrouracil angegeben ist[1]).

0.1146 g Sbst.: 0.1790 g CO_2, 0.0570 g H_2O.

$C_4H_6N_2O_2$. Ber. C 42.10, H 5.26.

Gef. » 42.58, » 5.51.

Das Filtrat vom Dihydrouracil wurde mit 100 ccm Wasser verdünnt und mit 1 g Natriumacetat und 3 g Phenylhydrazin in Essigsäure versetzt. Beim Erhitzen auf dem Wasserbade begann bald die Ausscheidung des Osazons, dessen Menge nach 3 Stunden 1,6 g betrug.

Nach dem Umlösen aus pyridinhaltigem Wasser schmolz das Osazon bei 163—164°. Es drehte in 10-prozentiger pyridin-alkoholischer Lösung in 0.5-dm-Rohr bei Natriumlicht 42° (± 0.5°) nach links: $\alpha_D = -84°$[2]).

0.1745 g Sbst.: 26.0 ccm N (22°, 760 mm).

$C_{17}H_{20}N_4O_3$. Ber. N 17.07. Gef. N 16.85.

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[1]) M. **17**, 172; B. **34**, 3759 [1901]. [2]) B. **42**, 1200 [1909].

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Weitere Versuche über die Entgiftung von Säure durch Salze.

Von

Jacques Loeb und Hardolph Wasteneys.

(Aus dem Rockefeller Institute, New York.)

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1. Die folgenden Tatsachen sollen zur Ergänzung einer früheren Mitteilung über denselben Gegenstand dienen[1]). Wir bestimmten die maximale Menge HCl, die durch verschiedene Konzentrationen einer Salzlösung noch entgiftet werden kann. Zunächst wurde zur Entgiftung eine Mischung von NaCl, KCl und $CaCl_2$ gewählt, und zwar in dem Verhältnis, in dem diese Salze im Seewasser existieren. Die folgenden zwei Beispiele sollen illustrieren, wie der Grenzwert für die entgiftende Wirkung erlangt wurde. Wie gewöhnlich wurden je 6 Fische (Fundulus) in 500 ccm der Lösung gebracht und jeden Tag die Zahl der überlebenden Fische ermittelt.

Tabelle I.

Nach	Zahl der überlebenden Fische in 100 ccm $^m/_2$-NaCl, KCl, $CaCl_2$ + 0,2	0,4	0,6	0,8	1,0 ccm $^n/_{10}$-HCl
1 Tage	6	6	4	0	0
4 Tagen	6	3	4		
11 „	5	2	4		

100 ccm $^m/_2$-Lösung von NaCl, KCl, $CaCl_2$ sind also im stande 0,6, aber nicht 0,8 ccm $^n/_{10}$-HCl zu entgiften.

[1]) Diese Zeitschr. 33, 489, 1911.

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Tabelle II.

Nach	Zahl der überlebenden Fische in 100 ccm $^{m}/_{4}$-NaCl, KCl, $CaCl_2$				
	+ 0,6	0,8	1,0	1,2	1,4 ccm $^{n}/_{10}$-HCl
1 Tage	6	6	6	5	1
4 Tagen	6	4	6	4	1
8 „	6	4	0	4	1

100 ccm $^{m}/_{4}$-NaCl, KCl, $CaCl_2$ sind also noch imstande, 1,4 ccm $^{n}/_{10}$-KCl zu entgiften. Der Grenzpunkt der Wirksamkeit läßt sich für die Entgiftung der Säure deshalb sicher bestimmen, weil, wenn die Entgiftungsgrenze einer Salzlösung für eine Säure überschritten ist, die Fische gewöhnlich schon in wenigen Stunden getötet werden.

Derartige Versuche wie in Tabelle I und II wurden in großer Zahl durchgeführt, um die entgiftende Wirkung verschiedener Konzentrationen von NaCl + KCl + $CaCl_2$ für HCl zu ermitteln. Tabelle III gibt das Resultat.

Tabelle III.

Konzentration der Lösung von NaCl + KCl + $CaCl_2$	Maximale Menge $^{n}/_{10}$-HCl, die die Fische in 100 ccm dieser Lösungen ertragen
0	0,1—0,2 ccm
$^{m}/_{100}$	0,2 „
$^{m}/_{16}$	0,5 „
$^{m}/_{8}$	1,0 „
$^{m}/_{4}$	1,2—1,4 „
$^{3}/_{8}$ m	0,8 „
$^{m}/_{2}$	0,6 „
$^{5}/_{8}$ m	0,3 „
$^{6}/_{8}$ m	0,2 „

Diese Zahlen haben vielleicht eine Bedeutung für die Frage, ob das Ödem der Zellen durch Säure bestimmt ist, eine Möglichkeit, auf die Loeb vor 14 Jahren die Aufmerksamkeit lenkte[1]). Man muß nun berücksichtigen, daß es sich bei vielen Formen des Ödems um einen Wasseraustritt in die Intercellularräume handelt und nicht um eine Schwellung der Zellen. Allein die hohe Menge Säure, die durch bloße Gegenwart von Neutralsalzen entgiftet wird, macht es zweifelhaft, ob die in den Zellen, namentlich bei Sauerstoffmangel, gebildete Säuremenge aus-

[1]) Loeb, Arch. f. d. ges. Physiol. 70, 467, 1898.

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reicht, um ein Ödem der Zellen hervorzurufen. Freilich ist die Möglichkeit zu berücksichtigen, daß die Konzentration der Salze in der Zelle niedriger ist als im Blute.

Man sieht, daß das Maximum der Säure durch eine $^m/_4$-Lösung entgiftet wird. Das ist ungefähr die Konzentration des Blutes mariner Teleostier. Fundulus lebt in einer $^m/_2$-Lösung (Seewasser), und wir erwarteten, daß auch eine solche Lösung die höchste Menge Säure zu entgiften imstande sein würde, was aber nicht der Fall ist.

2. Nach den Versuchen von Pauli und Handovsky über den Antagonismus zwischen Salzen und Säuren in bezug auf die physikalischen Eigenschaften der Eiweißkörper war zu erwarten, daß die entgiftende Wirkung einer reinen NaCl-Lösung und einer reinen $CaCl_2$-Lösung auf Säure für die Fische ungefähr die gleiche sein sollte. Das hat sich auch bestätigt, wie folgende Tabelle zeigt:

Tabelle IV.

Konzentration	Maximale Menge $^n/_{10}$-HCl, die durch 100 ccm entgiftet wird	
	NaCl	$CaCl_2$
$^m/_{500}$		0,2
$^m/_{100}$		0,3
$^m/_{50}$		0,4
$^m/_{16}$	0,4	0,5
$^m/_8$	0,8—1,0	0,8
$^m/_4$	1,2—1,4	
$^3/_8$ m	0,8	
$^m/_2$	0,5	

Man sieht, daß für sehr schwache Konzentrationen die entgiftende Wirkung von $CaCl_2$ etwas größer ist als die von NaCl (oder auch NaCl + KCl + $CaCl_2$), was wir ja schon früher fanden. Aber für Konzentrationen von $^m/_8$ ist die entgiftende Wirkung von $CaCl_2$ nicht größer als die von NaCl. Das entspricht dem Befunde von Pauli und Handovsky und stützt die Annahme, daß es sich bei der von uns bei Fischen gefundenen Entgiftung von Säuren durch Salze darum handelt, daß eine Säurewirkung auf Eiweiß durch Salze gehemmt wird.

Über die entgiftende Wirkung von Lösungen von $CaCl_2$, die stärker sind als $^m/_8$, konnten wir keine Versuche anstellen,

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weil in dieser Konzentration das $CaCl_2$ selbst die Fische zu rasch tötet.

3. Die direkte Beobachtung der Wirkung auf die Haut und die Kiemen gibt einige Aufschlüsse über den Mechanismus der schädlichen Wirkung der Säure und der Entgiftung derselben durch Salze. Werden die Fische in eine reine Säurelösung gebracht oder eine Säurelösung, die nicht genug Salz enthält, so wird die Haut bald weiß und fällt in Fetzen ab. Sobald aber die zur Entgiftung ausreichende Menge Salz zugefügt wird, hört diese Erscheinung ganz oder zum Teil auf, und die Haut bleibt völlig oder nahezu normal. Schneidet man die Kiemen aus einem Fundulus und bringt man dieselben in eine neutrale Lösung von $^m/_8$-NaCl + KCl + $CaCl_2$, so bleiben dieselben stundenlang normal. Bringt man die Kiemen in eine Lösung von 0,6 ccm $^n/_{10}$-HCl in destilliertem Wasser, so quellen dieselben rasch und werden trübe. Fügt man der Lösung aber genug NaCl zu, um die Säuren zu entgiften, so bleiben auch die Kiemen in einer solchen Lösung lange normal.

Wir deuteten bereits in der früheren Mitteilung an, daß der rasche Tod der Fische in einer Säurelösung dadurch herbeigeführt wird, daß die Kiemen zur Atmung unbrauchbar werden. Wie das geschieht, wird aus diesen direkten Beobachtungen an der Haut und den Kiemen klar. Die Epithelzellen werden durch die Säure zum Gerinnen gebracht. Ist die genügende Menge Neutralsalz zugegen, so benehmen sich die Zellen als ob die Säure nicht vorhanden oder wenigstens stark abgeschwächt wäre. Will man sich eine Vorstellung davon machen wie das möglich ist, so kann man vielleicht von der Erfahrung ausgehen, daß Säure Gelatine zum Schwellen bringt, während der ausreichende Salzzusatz die Gelatine entwässert. Nimmt man an, daß an der Oberfläche der Epithelzellen eine Schicht eines Eiweißkörpers vorhanden ist, der sich wie Gelatine verhält, so wird derselbe bei Gegenwart von Säure ohne Salz oder mit zu wenig Salz quellen und damit der Säure den Eintritt in die Zelle erlauben. Die in die Zelle eintretende Säure bedingt hier Gerinnung und damit den Tod der Zellen. Ist aber die genügende Menge Neutralsalz zugegen, so wird die Gelatine entwässert — gegerbt — und relativ undurchgängig für Säure. Die Säure bleibt in der Gelatine und gelangt nicht in die Zelle.

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Die Zellen der Kiemen und der Haut bleiben so am Leben.

4. Diese Tatsachen erlauben auch den Nachweis, daß Kohlensäure auf die Haut und die Kiemen ebenso wirkt wie jede andere Säure. Kohlensäure unterscheidet sich in ihrer Wirkung auf lebende Zellen von den starken Säuren wie HCl, daß die letzteren nur oder fast nur oberflächlich wirken, während CO_2 (infolge ihrer schwachen Dissoziation?) rasch in die Gewebe eindringt und so auch auf das Zentralnervensystem der Fische wirken kann. Läßt man einen kräftigen Strom von CO_2 ein paar Minuten durch destilliertes Wasser gehen und bringt man dann Fundulen in diese Lösung, so werden dieselben bald betäubt; etwas später aber stellen sich auch die vorhin geschilderten Säurewirkungen auf die Haut und die Kiemen ein. Bei der Anwendung von HCl bleiben die nervösen Wirkungen aus, weil diese Säure vornehmlich nur auf das Epithel wirkt, aber nicht durch die Haut eindringt.

Man kann nun leicht zeigen, daß Zusatz von Neutralsalz die oberflächliche Ätzwirkung der CO_2 auf die Haut der Fische aufzuheben, resp. zu verzögern imstande ist.

Wir verfuhren bei diesen Versuchen folgendermaßen: In einem Gasometer befand sich ein Gemisch von Luft und CO_2 in ganz bestimmtem Verhältnis. Mit diesem Gasometer waren 5 Glaszylinder von ca. 200 ccm Volumen verbunden, in die 50 ccm Flüssigkeit gebracht wurden. Jeder Zylinder hatte einen luftdicht eingeschliffenen Glasdeckel, in den zwei Glasröhren eingeschmolzen waren, eine zum Einführen, die andere zum Ausführen der Gase. Jede Röhre war mit einem luftdicht schließenden Einweghahn versehen. Wenn die Durchströmung beendet war, so konnte man durch Schließen der Hähne jeden Zylinder luftdicht abschließen. Vor Beginn des Versuches wurde $^1/_2$ Stunde lang ein sehr kräftiger Strom des Luft-CO_2-Gemisches durch alle 5 Gefäße geleitet. Dann wurden in jedes Gefäß je 2 kleine Fische gebracht, während der Strom noch durchging, und nach dem Einbringen der Fische (Fundulus) wurde der Strom noch 5 Minuten lang unterhalten. Dann wurde jedes Gefäß durch Abschluß der Hähne isoliert. Jedes Gefäß enthielt genug O, um die Fische 2 Tage bei der Temperatur der Versuche (ca. 19°) am Leben zu erhalten. Wir wollen nun die

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Resultate einer Reihe von Versuche beschreiben, die mit verschiedener CO_2-Tension angestellt wurden. In der ersten Versuchsreihe enthielten die 5 Versuchsflaschen je 50 ccm H_2O, $^m/_{50}$-, $^m/_{25}$-, $^m/_{10}$-, $^m/_5$-NaCl. Der Gehalt an Luft + CO_2 ist in Volumenprozent (bei Atmosphärendruck) angegeben. Die Temperatur war in allen Versuchen 18 bis 19°, ging in der Nacht aber etwas herunter.

Jede Lösung enthielt 2 Fische.

Tabelle V.

Gasgemisch	Zahl der überlebenden Fische nach 18 Std. Natur der Lösung				
	H_2O	$^m/_{50}$-	$^m/_{25}$-	$^m/_{10}$-	$^m/_5$-NaCl
5% CO_2, 95% Luft	1	1	2	2	2
10% CO_2, 90% Luft	0	2	2	2	2
15% CO_2, 85% Luft	0	0	2	2	2
20% CO_2, 80% Luft	0	0	0	2	2
25% CO_2, 75% Luft	0	0	0	0	1
$33^1/_3$% CO_2, $66^2/_3$% Luft	0	0	0	0	0

Kontrollversuche, in denen die Ordnung der Gefäße eine andere war, gaben dasselbe Resultat. Bei den Fischen, die in H_2O oder in den schwachen Salzlösungen waren, wurde die Haut der Flossen und des ganzen Körpers rasch weiß. Bei stärkeren Konzentrationen der CO_2 trat das auch in den schwächeren Salzlösungen ein.

Da es wegen der Giftigkeit reiner Chlornatriumlösungen nicht möglich war, höhere Konzentrationen des Salzes anzuwenden, wurden noch einige Versuche mit einem Gemisch von NaCl + KCl + $CaCl_2$ angestellt in dem Verhältnis, in dem diese Salze im Seewasser enthalten sind.

Tabelle VI.

Gasgemisch	Zahl der überlebenden Fische nach 18 Std. Natur der Lösung				
	H_2O	$^m/_8$-	$^m/_4$-	$^3/_8$ m-	$^m/_2$-NaCl, $CaCl_2$, KCl
20% CO_2, 80% Luft	0	2	2	2	0
25% CO_2, 75% Luft	0	0	1	1	

Man sieht wieder, daß eine $^m/_2$-Lösung keine so starke entgiftende Wirkung hat wie eine $^m/_4$-Lösung. Die Resultate sind in Übereinstimmung mit den in Tabelle V erhaltenen.

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Vor 13 Jahren wies Loeb in der Festschrift für A. Fick zuerst darauf hin, daß ein fundamentaler Unterschied in der physiologischen Wirkung der Salze und Nichtleiter besteht, und später wies er nach, daß die Giftwirkung der Elektrolyte zwar durch gewisse andere Elektrolyte, aber im allgemeinen nicht durch Nichtleiter aufgehoben werden kann. Wir stellten Versuche über die Entgiftung von CO_2 und HCl mit Dextrose und Rohrzucker an, die aber alle negativ verliefen. Dextrose und Rohrzucker hemmen die giftige Wirkung von Säuren nicht oder kaum merklich.

Sumner behauptet, daß, wenn man Fundulus in ein Gefäß mit destilliertem Wasser bringt, die Fische rasch sterben (in etwa 2 bis 5 Tagen), daß aber der Zusatz von wenig Seewasser oder Salz sie am Leben erhält. Sumner brachte immer relativ viele Fische in wenig destilliertes Wasser. Wir finden aber, daß, wenn man nur wenig Fische in viel destilliertes Wasser bringt, dieselben am Leben bleiben. Wenn man viele Fische in eine kleine Menge destilliertes Wasser bringt, wie das Sumner in seinen Versuchen tat, so kommt es vermutlich zu einer langsamen Vergiftung der Fische durch die von ihnen produzierte CO_2 und andere Excrete, und diese Vergiftung kann dann durch Salzzusatz aufgehoben werden.

Es ist jedenfalls sicher, daß die Süßwasserorganismen wehrlos gegen die Säurewirkung der CO_2 sind, und es ist zu vermuten, daß das ein weiterer Umstand ist, der es bedingt, daß die Süßwasserfauna lange nicht so reich ist wie die Seewasserfauna.

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Über die Entgiftung von Natriumbromid.

Von

Jacques Loeb und Hardolph Wasteneys.

(Aus dem Rockefeller Institute, New York.)

(Eingegangen am 12. Januar 1912.)

1. Wir können zwischen artfremden und arteigenen Ionen unterscheiden. Wir wollen in dieser Arbeit die Frage aufwerfen, ob die Gesetze der Entgiftung artfremder Ionen mit denen für die Entgiftung arteigener Ionen identisch sind. Wir beginnen mit Versuchen an NaBr, wobei Br das artfremde Ion ist. Es eignet sich besonders gut für Entgiftungsversuche, da die Fische durch dasselbe rasch betäubt werden und auf der Seite oder dem Rücken liegen und schwimmen. Sie können tagelang in diesem betäubten Zustande liegen ehe sie sterben. Es handelt sich um eine Wirkung des Br auf das Zentralnervensystem, vielleicht auf die mit dem Acusticus zusammenhängenden Zentren. Da schon sehr geringe Dosen von NaBr diese Wirkung hervorrufen, so ist diese Substanz für die Entgiftungsversuche sehr geeignet.

Die Versuche wurden wieder an Fundulus angestellt, ein Fisch, der (wie nochmals erwähnt sein soll) im Seewasser sowohl wie im destillierten Wasser leben kann. Je 6 Fische wurden in 500 ccm der Lösung gebracht und jeden Tag die Zahl der überlebenden Fische festgestellt. Die Temperatur schwankte um 18°.

Die ersten zwei Tabellen sollen zeigen, bis zu welchem Grade die Entgiftung möglich ist. Die eine Tabelle gibt das Verhalten der Fische in verschiedenen Konzentrationen einer reinen Natriumbromidlösung; die zweite Tabelle gibt das Ver-

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halten der Fische in Bromnatriumlösungen derselben Konzentration, nur mit dem Unterschied, daß die Lösung in $^m/_8$-NaCl + $CaCl_2$ + KCl statt in destilliertem Wasser angefertigt war. (Die drei Salze waren im üblichen Verhältnis von 100 Molekülen NaCl, 1,5 Molekülen $CaCl_2$, 2,2 Molekülen KCl.) Die Tiere blieben in der letzteren Lösung nicht nur viel länger am Leben, sie blieben auch gesund, während sie in den reinen Bromnatriumlösungen meist auf der Seite lagen, also die Bromvergiftung zeigten. Um nun in der Tabelle nicht nur die Zahl der überlebenden, sondern auch die relative Zahl der gesunden und erkrankten Fische erkennen zu lassen, bedienen wir uns folgender Darstellung. Wenn in einer Lösung 6 Fische am Leben, aber 2 gesund und 4 erkrankt sind, so wird in der Tabelle die Zahl der überlebenden Fische als eine Summe dargestellt, wobei der erste Summand die gesunden, der zweite die kranken Fische bezeichnet; sind also 2 gesund und 4 krank, so wird das Resultat in der Form 2 + 4 ausgedrückt. Sind 4 gesund und 2 krank, so schreiben wir 4 + 2. Sind alle überlebenden Fische gesund, so schreiben wir bloß eine Zahl; sind alle überlebenden Fische, sagen wir 3, krank, so schreiben wir 0 + 3. Wo also die Zahl der überlebenden Fische durch eine Summe ausgedrückt ist, bedeutet der zweite Summand immer die Zahl der auf der Seite liegenden Fische, die die Bromvergiftung zeigen.

Tabelle I[1]).

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-NaBr in 100 ccm H_2O						
	2,0	4,0	8,0	16,0	20,0	25,0	30,0
1	6	6	6	6	4 + 2	3 + 3	3 + 3
2	6	5 + 1	3 + 3	6	3 + 3	0 + 6	2 + 3
4	4 + 2	1 + 4	1 + 5	2 + 2	0 + 1	0 + 4	0 + 1
5	3 + 3	1 + 3	2 + 2	1 + 0	0	0	0 + 1
6	0	0	0	0	—	—	0

[1]) In bezug auf die Natur der Lösungen sei folgendes bemerkt. 30 ccm $^m/_2$-NaBr in 100 ccm H_2O bedeutet, daß zu 30 ccm $^m/_2$-NaBr 70 ccm H_2O zugesetzt wurden. 30 ccm $^m/_2$-NaBr in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$ bedeutet, daß zu 30 ccm $^m/_2$-NaBr so viel der Mischung von NaCl + KCl + $CaCl_2$ zugesetzt wurde, daß die Lösung in bezug auf die letztere Mischung $^m/_8$ war.

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Man sieht also, daß in einer Lösung von 2,0 ccm $^m/_2$-NaBr in 100 ccm H_2O nach 4 Tagen bereits 2 Fische erkrankt sind und daß nach 6 Tagen alle Fische tot sind. In einer Mischung von 20 ccm $^m/_2$-NaBr + 80 ccm H_2O zeigten bereits nach 24 Stunden 2 Fische die charakteristischen Symptome der NaBr-Vergiftung. Die niedrige Konzentration der Lösungen war nicht für das Resultat verantwortlich, da die Fische in Lösungen von NaCl von derselben Konzentration dauernd am Leben bleiben.

Gleichzeitig mit diesem Versuche, d. h. mit demselben Material und derselben Temperatur wurde eine korrespondierende Versuchsreihe angestellt, in der die Konzentrationen des NaBr genau dieselben waren; nur wie schon erwähnt, daß das Lösungsmittel nicht reines Wasser, sondern eine $^m/_8$-Lösung von NaCl + $CaCl_2$ + KCl war. Der entgiftende Einfluß dieser Lösung wird aus Tabelle II ohne weiteres deutlich.

Tabelle II.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-NaBr in 100 ccm $^m/_8$-NaCl + $CaCl_2$ + KCl						
	2,0	4,0	8,0	16,0	20,0	25,0	30,0
1	6	6	6	6	6	6	6
2	6	6	6	6	5 + 1	6	6
4	6	6	6	6	5	6	6
5	6	6	6	6	5	6	6
6	6	6	6	6	5	6	6

Vergleicht man Tabelle II mit Tabelle I, so ergibt sich, daß nach 6 Tagen, als in der reinen NaBr-Lösung alle Fische an Bromvergiftung gestorben, dieselben in derselben Lösung von NaBr durch den Zusatz von NaCl, KCl und $CaCl_2$ alle (mit Ausnahme von einem Fisch) am Leben erhalten und von der Bromvergiftung frei waren.

Will man das Resultat nach der Durchlässigkeitstheorie erklären, so muß man sagen, daß der Zusatz von NaCl + KCl + $CaCl_2$ die Haut und Kiemen der Fische für die hier benutzten Dosen von NaBr dauernd oder für lange Zeit undurchgängig machte. Die Fische der Tabelle II starben schließlich auch, aber fast alle ohne Symptome der Bromvergiftung.

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2. Es läßt sich leicht zeigen, daß NaBr von nicht zu hoher Konzentration auch durch eine reine NaCl-Lösung entgiftet werden kann; und daß der Entgiftungskoeffizient NaBr/NaCl nahezu gleich 1 ist, d. h. daß der Zusatz der nahezu gleichen molekularen Menge von NaCl zu NaBr ausreicht, die Entgiftung von NaBr sicher herbeizuführen. Von den sehr zahlreichen Versuchen sollen 4 zur Erläuterung mitgeteilt werden; nämlich für 1, 4, 8 und 20 ccm $^m/_2$-NaBr in 100 ccm der Lösung.

Tabelle III.

Nach Tagen	Zahl der überlebenden Fische in 1 ccm $^m/_2$-NaBr in 100 ccm H_2O + ccm $^m/_2$-NaCl								
	0	0,1	0,2	0,4	0,6	0,8	1,0	1,2	1,4
2	5+1	5+1	5+1	6	6	6	6	6	6
4	2+3	2+1	2+3	3+3	3+2	6	6	5	6
6	0+4	0+2	0+1	2+3	4	5+1	5	5	5

Die Fische, die 1,0 oder mehr $^m/_2$-NaCl enthielten, blieben von der Bromvergiftung dauernd oder wenigstens sehr lange verschont.

Tabelle IV.

Nach Tagen	Zahl der überlebenden Fische in 4,0 ccm $^m/_2$-NaBr + ccm $^m/_2$-NaCl in 100 ccm H_2O						
	0,5	1,0	2,0	4,0	6,2	12,5	18,7
3	3+3	4+2	5+1	6	6	6	6
5	0	2+3	3+3	6	6	6	6
7	—	0+4	1+5	5	6	5	6

Die Fische, die in Lösungen waren, die 4 ccm oder mehr NaCl enthielten, blieben von der NaBr-Vergiftung dauernd oder sehr lange verschont.

Tabelle V.

Nach Tagen	Zahl der überlebenden Fische in 8 ccm $^m/_2$-NaBr + ccm $^m/_2$-NaCl in 100 ccm H_2O							
	1,0	2,0	3,0	4,0	5,0	6,0	8,0	10,0
3	1+5	5+1	3+3	4+2	5+1	6	6	6
6	0+5	0+2	3+3	2+3	5	6	4+1	5
12	0	1+1	0+1	0	5	5	3+1	4

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Die Entgiftung trat hier beim Zusatz von 5,0 ccm $^m/_2$-NaCl ein, war aber beim Zusatz von 8 ccm $^m/_2$-NaCl offenbar nicht vollständig; war aber vollständig bei Zusatz von 10,0 ccm $^m/_2$-NaCl.

Tabelle VI.

Nach Tagen	Zahl der überlebenden Fische in 20 ccm $^m/_2$-NaBr + 0	5,0	10,0	15,0	20,0	25,0 ccm $^m/_2$-NaCl
3	0+2	3+2	4+1	6	5	6
6	0	0+3	3+2	2	3	5

Die schützende Wirkung des NaCl trat in diesem Falle noch nicht bei Zusatz von 10,0 ccm $^m/_2$-NaCl, wohl aber bei Zusatz von 15,0 ccm oder mehr NaCl ein.

Aus diesen und vielen anderen Versuchen dürfen wir schließen, daß der Entgiftungskoeffizient von NaBr/NaCl etwa ungefähr gleich 1, oder ein wenig größer als 1 ist.

3. Es war von großem Interesse, den Entgiftungskoeffizienten von NaBr durch $CaCl_2$ zu bestimmen. Die Versuche geben den Eindruck, als ob $CaCl_2$ ungefähr zweimal so wirksam für die Entgiftung von NaBr ist als NaCl.

Tabelle VII.

Nach Tagen	Zahl der überlebenden Fische in 2 ccm $^m/_2$-NaBr in 100 ccm H_2O + 0	0,1	0,4	0,8	1,0	1,5	2,0 ccm $^m/_2$-$CaCl_2$
4	0+1	0+2	0+6	6	6	4	5
7	0+1	0	3+1	2+2	6	4	5
11	0+1		3+1	2+2	6	4	5

1,0 ccm $^m/_2$-$CaCl_2$ entgiftet also 2 ccm $^m/_2$-NaBr in 100 ccm H_2O.

Tabelle VIII.

Nach Tagen	Zahl der überlebenden Fische in 4 ccm $^m/_2$-NaBr in 100 ccm H_2O + 0,5	1,0	2,0	4,0	6,2 ccm $^m/_2$ $CaCl_2$
4	6+1	6	6	6	6
7	1+5	2+4	6	6	6
11	0+3	4+1	5	5	5

4 ccm $^m/_2$-NaBr werden also durch 2,0 ccm $^m/_2$-$CaCl_2$ entgiftet.

In derselben Weise wurde ermittelt, daß 8 ccm $^m/_2$-NaBr in 100 ccm H_2O durch 4 bis 5 ccm $^m/_2$-$CaCl_2$ entgiftet werden.

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Vergleicht man diese Zahlen mit den für die Entgiftung von NaBr durch NaCl gefundenen Zahlen, so gewinnt man den Eindruck, daß $CaCl_2$ ungefähr zweimal so stark entgiftend wirkt wie NaCl. Dieses Resultat erweckt den Eindruck, als ob in diesem Falle das Cl-Ion das entgiftende Ion sei. Diese Ansicht erhält vielleicht eine Stütze durch die Tatsache, daß die entgiftende Kraft von $MgCl_2$ auf NaBr ungefähr ebenso groß ist wie die entgiftende Kraft von $CaCl_2$, während die entgiftende Kraft von $MgCl_2$ auf KCl sowohl wie auf NaCl sehr gering ist[1]). Da in diesen beiden letzteren Fällen die giftige sowohl wie die entgiftende Wirkung vom Kation ausgeht, so scheint das die Vermutung zu stützen, daß das entgiftende Anion bei der Entgiftung von Brom das Cl-Ion ist.

Es lag im Anschluß an diese Beobachtungen nahe, Versuche darüber anzustellen, ob $CaBr_2$ ebenfalls imstande ist, eine NaBr-Lösung zu entgiften. Das ist nicht der Fall, wie folgende Tabelle zeigt.

Tabelle IX.

Nach Tagen	Zahl der überlebenden Fische in 4 ccm $^m/_2$-NaBr in 100 ccm H_2O					
	+ 0	0,8	1,6	2,0	3,0	4,0 ccm $^m/_2$-$CaBr_2$
3	1 + 4	2 + 4	6	5 + 1	3 + 3	3 + 3
7	0 + 4	1 + 1	0 + 3	0 + 3	0 + 3	0 + 5
12	0 + 3	0 + 2	0 + 1	0 + 3	0	0 + 4

Man sieht, daß in den Lösungen ohne $CaBr_2$ die Giftwirkung des Br nicht stärker ist als in den Lösungen mit Zusatz von $CaBr_2$. Ein gleichzeitig angestellter Parallelversuch mit $CaCl_2$ ergab im Gegensatz zu diesem Versuch sehr starke entgiftende Wirkung.

Tabelle X.

Nach Tagen	Zahl der überlebenden Fische in 4 ccm $^m/_2$-NaBr in 100 ccm H_2O				
	+ 1,0	1,5	2,0	3,0	4,0 ccm $^m/_2$-$CaCl_2$
3	5 + 1	6	5	6	5
7	1 + 3	1 + 5	4	6	4
12	0	0 + 2	2	6	4
17		0	0	6	4

[1]) Diese Zeitschr. 31, 450, 1911; 32, 308, 1911.

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In den Lösungen mit 2,0 ccm $^m/_2$-$CaCl_2$ oder darüber trat keine Br-Vergiftung der Fische ein. Im übrigen bestätigt diese Tabelle das schon früher über die entgiftende Wirkung von $CaCl_2$ Gesagte.

Alle diese Versuche finden eine einfache Erklärung, wenn man die entgiftende Wirkung dem Cl-Ion zuschreibt. Bei der Entgiftung von NaBr durch NaCl dürfte ja hierüber kein Zweifel bestehen. Wir müssen aber auch eine Tatsache berichten, die dieser Annahme eine Schwierigkeit bereitet, nämlich daß $CaBr_2$ viel weniger giftig ist als NaBr. Die folgenden zwei Versuche, die gleichzeitig angestellt wurden, zeigen das sehr deutlich.

Tabelle XI.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-NaBr in 100 ccm H_2O						
	2,0	6,0	10,0	14,0	20,0	28,0	36,0
3	4+2	0+3	0	0+4	0+2	0+3	0+1
5	0	0		0	0	0	0

Tabelle XII.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$CaBr_2$ in 100 ccm H_2O						
	2,0	6,0	10,0	14,0	20,0	28,0	36,0
3	6	6	6	4+1	5	1+1	0
5	6	6	6	4	5	0+2	
10	5	3	1+4	1+2	0+1	0	
15	1+1	0	0	1	0+1		

Man sieht, daß die Fische in Calciumbromidlösungen länger von den Symptomen der Bromvergiftung verschont und am Leben bleiben als in Natriumbromidlösungen von derselben molekularen Konzentration; obwohl doch in den aquimolekularen Calciumbromidlösungen die Konzentration der Br-Ionen fast doppelt so hoch ist wie in den Natriumbromidlösungen. Die Tatsache wird verständlich und verliert alles Widerspruchsvolle, wenn wir annehmen, daß nicht die Ionen, sondern die Salzmoleküle in den Fisch eindringen und daß die $CaBr_2$-Moleküle langsamer eindringen als die NaBr-Moleküle, was ja dem allgemeinen Verhalten der Calciumsalze entspricht.

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Es muß auch bemerkt werden, daß die Lösungen von Natriumbromid, die Calcium in ausreichender Menge enthalten, länger klar bleiben als die Lösungen von Natriumbromid ohne Calcium.

4. Die bisher erwähnten Versuche weisen darauf hin, daß die Cl-Ionen der Chloride die Entgiftung der Bromide herbeiführen, während die Kationen der entgiftenden Salze vielleicht nur indirekt dabei in Betracht kommen. Diese Ansicht läßt sich einer weiteren Prüfung unterziehen, nämlich indem wir andere Salze in bezug auf ihre entgiftende Wirkung auf Natriumbromid prüfen. Diese Versuche ergaben das Resultat, daß Na_2SO_4 und $NaHCO_3$ die Giftigkeit der Bromsalze in keinem Falle zu hemmen oder auch nur zu verringern imstande sind. Eher ließe sich das Gegenteil behaupten. Es handelt sich also wohl um eine spezifische Funktion des Cl bei dieser Entgiftung. Es sei schließlich auch noch bemerkt, daß es nicht gelang, mit KCl eine Entgiftung von NaBr herbeizuführen, weil KCl in den Konzentrationen, in denen Cl das NaBr zu entgiften imstande wäre, die Tiere rasch tötet. Aus demselben Grunde erwies sich auch LiCl als unbrauchbar.

5. Man kann zuletzt die Frage aufwerfen, welches die maximale Konzentration von NaBr ist, die sich entgiften läßt. Während schon eine reine $^m/_{200}$ Lösung von NaBr die typischen Symptome der Bromvergiftung hervorruft, ist in einer $^m/_8$ Lösung von NaCl + KCl + $CaCl_2$ selbst eine $^m/_{10}$ oder $^m/_8$ Lösung von NaBr fast harmlos. Die maximale Konzentration von NaBr, die durch NaCl entgiftet werden kann, ist niedriger als die maximale Konzentration, die durch NaCl + KCl + $CaCl_2$ entgiftet werden kann, wenn diese drei Salze in dem Verhältnis zugesetzt werden, in dem sie im Seewasser vorhanden sind.

6. Die Theorie der Giftwirkung sowie der Entgiftung von NaBr ist analog derjenigen, die wir für die Giftwirkung und Entgiftung von KCl aufgestellt haben. Nehmen wir an, daß eine gewisse Zahl von Eiweißmolekülen an der Oberfläche der Kiemen des Fisches bereit sind, sich mit NaBr zu verbinden, so tritt die Giftwirkung ein, sobald etwa $^1/_3$ dieser Eiweißmoleküle sich mit NaBr verbindet. Ist genug NaCl oder $CaCl_2$ vorhanden, so daß über $^2/_3$ der für Br zugänglichen Bindungen durch Cl ersetzt werden können, so tritt keine Bromvergiftung

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ein. Wie im Falle der Entgiftung von Kaliumsalzen die entgiftende Wirkung dem Kation des entgiftenden Salzes zugeschrieben werden mußte, so muß bei der Entgiftung von Br die entgiftende Wirkung dem Anion Cl zugeschrieben werden.

Zusammenfassung der Ergebnisse.

1. Es wird gezeigt, daß bereits eine sehr schwache Konzentration von NaBr ($^{m}/_{200}$) eine typische Bromvergiftung der Fische hervorruft.

2. Es wird ferner gezeigt, daß, sobald zweimal so viel Cl-Ionen in der Volumeneinheit der Lösung sind als Br-Ionen, die Lösung entgiftet wird; gleichviel ob diese Cl-Ionen durch NaCl, $CaCl_2$ oder $MgCl_2$ geliefert werden.

3. Wie im Falle der Entgiftung von KCl die entgiftende Wirkung dem Kation des entgiftenden Salzes zugeschrieben werden mußte, so muß hier wohl die entgiftende Wirkung dem Anion (Cl) des entgiftenden Salzes zugeschrieben werden.

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Sonderabdruck aus d. Biochemischen Zeitschrift 39. Bd., S. 194.

Die Abhängigkeit der relativen Giftigkeit von Na und Ca von der Natur des Anions.

Von

Jacques Loeb.

(Aus dem Rockefeller Institute, New York.)

(Eingegangen am 18. Januar 1912.)

Es wird im allgemeinen angenommen, daß die Wirkung eines Salzes eine additive Funktion seiner Ionen ist. Wenn diese Annahme richtig ist, so muß die relative Giftigkeit der Salze zweier Kationen stets für jedes beliebige Anion dieselbe sein. Eine Prüfung dieses Satzes ist dadurch erschwert, daß im allgemeinen lebende Organismen so sehr vom osmotischen Druck abhängen, daß man nicht mit verschiedenen Konzentrationen eines Salzes Versuche anstellen kann, ohne eine zweite Substanz zuzusetzen, die den osmotischen Druck immer auf demselben Niveau hält. Von diesem Übelstand sind wir bei Fundulus frei, und so habe ich eine Reihe derartiger Versuche ausgeführt. Es wird allgemein angegeben, daß eine reine Lösung von $CaCl_2$ viel giftiger ist als eine reine NaCl-Lösung. Das bestätigt sich auch für Fundulus. In einer $^2/_5$ m-Lösung von $CaCl_2$ sterben die Fische schon in etwa einer Stunde, und selbst in einer $^m/_4$-Lösung von $CaCl_2$ leben sie weniger als 18 Stunden (bei etwa 18° C). Den Unterschied in der relativen Giftigkeit von $CaCl_2$ und NaCl bringt folgende Tabelle zum Ausdruck. Die beiden Versuchsreihen wurden gleichzeitig angestellt. Am Anfang enthielt jede Lösung 6 Fische (in 500 ccm der Lösung).

Tabelle I.

Nach	Zahl der überlebenden Fische in 30 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O	40	50	60	80	90
2 Stunden	6	6	4	2	0	0
18 „	6	4	0	0		
2 Tagen	5	3				
3 „	5	2				
5 „	5	0				
8 „	2					

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Tabelle II.

Nach	Zahl der überlebenden Fische in ccm $^m/_2$-NaCl in 100 ccm H_2O					
	30	40	50	60	80	90
1 Tage	6	6	6	6	6	6
2 Tagen	6	6	6	5	6	6
5 „	6	4	6	4	0	0
8 „	6	4	4	1		

Ein Vergleich der Tabellen lehrt, daß in 60 ccm $^m/_2$-NaCl die Lebensdauer ungefähr die gleiche ist wie in 30 ccm $^m/_2$-$CaCl_2$. Man kann sagen, daß $CaCl_2$ für Fundulus ungefähr zweimal so giftig ist wie NaCl.

Vergleicht man aber die relative Giftigkeit von NaBr und $CaBr_2$, so findet man das entgegengesetzte Verhalten; NaBr ist viel giftiger als $CaBr_2$. Es ist aber nicht gut möglich, die relative Giftigkeit in einem bestimmten Zahlenverhältnis anzugeben. Ein Blick auf die folgenden Tabellen wird das Gesagte klar machen. Um die Verhütung der Bromvergiftung durch Ca wollen wir uns hier nicht kümmern, sondern nur die Zahl der Überlebenden als Maßstab nehmen.

Tabelle III.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-NaBr in 100 ccm H_2O				
	2,0	4,0	8,0	16,0	20,0
2	6	6	5	6	3
5	0	0	2	0	0
9			0		

Tabelle IV.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$CaBr_2$ in 100 ccm H_2O				
	2,0	4,0	8,0	16,0	20,0
2	6	6	5	4	5
5	6	6	3	1	4
9	5	6	3	1	1

Es ist nicht übertrieben, wenn man behauptet, daß 8 ccm $^m/_2$-NaBr ebenso giftig für Fundulus ist wie 20 ccm $^m/_2$-$CaBr_2$.

Dieses Beispiel zeigt also deutlich, daß die relative Giftigkeit zweier Kationen nicht konstant ist, wie man erwarten sollte, sondern daß dieselbe von dem Anion abhängt. Die

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relative Giftigkeit von Na und Ca kehrt ihr Vorzeichen um, wenn man Chloride und Bromide vergleicht! Das gab Veranlassung, die relative Giftigkeit von Na und Ca auch für einige andere Salze festzustellen.

Tabelle V und VI geben die Resultate von zwei gleichzeitig angestellten Versuchsreihen mit $NaNO_3$ und $Ca(NO_3)_2$.

Tabelle V.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$NaNO_3$ in 100 ccm H_2O				
	2,0	10,0	20,0	40,0	60,0
1	6	3	2	0	0
2	5	0	0		
3	1				
4	0				

Tabelle VI.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$Ca(NO_3)_2$ in 100 ccm HO_2				
	2,0	10,0	20,0	40,0	60,0
1	6	6	5	0	0
2	6	6	5		
3	6	3	1		
4	6	1	1		

Es geht aus dieser Tabelle hervor, daß $Ca(NO_3)_2$ bei weitem nicht so giftig für Fundulus ist wie $NaNO_3$.

Dasselbe ergab sich beim Vergleich der Giftigkeit von essigsaurem Natrium und Calcium (Tabelle VII und VIII).

Tabelle VII.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$NaCH_3COO$ in 100 ccm H_2O							
	2,0	8,0	12,0	16,0	20,0	25,0	30,0	40,0
3	5	4	3	5	4	3	3	0
5	2	2	1	0	1	1	0	
8	2	2	1		1	1		

Tabelle VIII.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$Ca(CH_3COO)_2$ in 100 ccm H_2O							
	2,0	8,0	12,0	16,0	20,0	25,0	30,0	40,0
3	5	6	6	6	5	4	5	5
5	5	3	6	6	5	2	2	0
8	4	3	6	6	2	2	1	

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Während die Lösungen von essigsaurem Natrium alsbald alle trübe wurden, blieben die Lösungen mit essigsaurem Calcium viel länger klar. Daß essigsaures Natrium giftiger ist als essigsaures Calcium, ist hinreichend deutlich.

Am schlagendsten fiel ein Vergleich der Giftigkeit von NaHO und $Ca(HO)_2$ auf Fundulus aus (Tabelle IX und X).

Tabelle IX.

Nach Tagen	Zahl der überlebenden Fische in ccm $^n/_{10}$-NaHO in 100 ccm H_2O					
	0	0,4	0,8	1,4	1,6	2,0
1	6	4	5	1	0	0
5	4	3	4	0		

Tabelle X.

Nach Tagen	Zahl der überlebenden Fische in ccm $^n/_{10}$-$Ca(HO)_2$ in 100 ccm H_2O					
	1,5	2,0	4,0	6,0	8,0	10,0
1	6	6	6	5	6	2
5	6	5	5	5	3	0

Die Giftigkeit von NaHO ist nahezu 10 mal so groß wie die Giftigkeit von $Ca(HO)_2$ bei gleicher Konzentration der Hydroxylionen. Mit der elektrolytischen Dissoziation hat dieses Resultat nichts zu tun, da beispielsweise in einer $^n/_{1000}$-Lösung beide Basen praktisch vollständig dissoziiert sind.

Diese Beispiele mögen genügen, um zu zeigen, daß für eine Reihe von Anionen die Na-Salze giftiger sind als die Ca-Salze; während für die Chloride das umgekehrte der Fall ist. Was bedingt diesen Einfluß des Anions auf die relative Giftigkeit der Na- und Ca-Salze? Man könnte daran denken, daß im Falle der artfremden Anionen die Ordnung der Giftigkeit geändert werde. Es war deshalb von Wichtigkeit, den Einfluß der SO_4-Ionen zu prüfen. Da $CaSO_4$ nicht löslich genug für einen solchen Vergleich ist, so wurde die relative Giftigkeit von Na_2SO_4 und $MgSO_4$ verglichen. Frühere Versuche hatten schon ergeben, daß $MgCl_2$ für diese Fische ein wenig giftiger ist als $CaCl_2$, daß es also sicher giftiger ist als NaCl. Für die Sulfate aber stellte es sich heraus, daß das

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umgekehrte der Fall ist, wie der in Tabelle XI und XII dargestellte Parallelversuch zeigt.

Tabelle XI.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-Na_2SO_4 in 100 ccm H_2O					
	2,0	8,0	16,0	20,0	25,0	30,0
1	5	6	6	2	2	0
3	4	5	0	0	0	
10	4	5				

Tabelle XII.

Nach Tagen	Zahl der überlebenden Fische in ccm $^m/_2$-$MgSO_4$ in 100 ccm H_2O					
	2,0	8,0	16,0	20,0	25,0	30,0
1	5	5	6	5	6	5
3	5	5	6	4	5	3
10	4	1	4	1	2	1

$MgSO_4$ ist also sicher weniger giftig als Na_2SO_4. Das SO_4-Ion kommt aber im Seewasser natürlicherweise vor. Auch $Mg(NO_3)_2$ ist weniger giftig als $NaNO_3$.

Die Erklärung ist in einer anderen Richtung zu suchen. Es ist möglich, daß sich die relative Giftigkeit verschiedener Ionen mit der Konzentration verschiebt. Von einer gewissen Konzentration an ist Ca immer giftiger als Na; unterhalb einer gewissen Konzentration ist die lebenserhaltende Wirkung von Ca größer als die von Na. Haben wir es mit Anionen zu tun, die an sich relativ giftig sind, wie im Falle von HO oder NO_3, so ist das Ca-Salz immer weniger giftig als das Na-Salz, aus dem einfachen Grunde, weil in diesen Fällen die Konzentration der Salze sich immer in den Grenzen bewegt, innerhalb derer Ca das Leben (d. h. die Integrität der Oberflächenschicht der Zelle) besser erhält als Na. Ist das Ion aber sehr ungiftig, wie im Falle von Cl, so hält das Tier eine so hohe Konzentration von NaCl aus, daß die Giftigkeitsgrenze von $CaCl_2$ weit überschritten wird.

Der Umstand, daß die Konzentration der Ionen für die relative Giftigkeit derselben in Betracht kommt, dürfte daher rühren, daß die Salze in erster Linie die Natur und Durch-

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lässigkeit der Oberflächenlamelle der Zellen bestimmen. Nicht nur der Mangel, sondern auch ein erheblicher Überschuß an Ca kann schädigend auf die Membran wirken.

Auch die relative Giftigkeit der Anionen sollte nach dem Gesagten sich mit der Natur des Kations ändern. Das scheint nach wenigen Versuchen, die ich bis jetzt habe anstellen können, auch der Fall zu sein.

Zusammenfassung der Ergebnisse.

Während allgemein angenommen wird, daß die Giftigkeit eines Salzes eine additive Funktion seiner Ionen ist, wird in dieser Arbeit gezeigt, daß sich die relative Giftigkeit von Na und Ca und von Na und Mg auf Fundulus mit der Natur des Anions ändert. NaCl ist nicht so giftig wie $CaCl_2$ oder $MgCl_2$; für eine Reihe von Anionen aber, nämlich Br, HO, NO_3, CH_3COO und SO_4 ist Ca resp. Mg weniger giftig als Na.

Es wird darauf hingewiesen, daß dieses Verhalten verständlich wird unter der Annahme, daß die relative Giftigkeit der Ionen sich mit der Konzentration derselben ändert; daß beispielsweise in niedriger Konzentration die schützende Kraft von Ca größer ist als die von Na; während von einer gewissen Konzentration an Na weniger schädlich ist als Ca. Ist nun das Anion so giftig, daß alle Versuche sich in relativ niedrigen Konzentrationen der Salze bewegen müssen, so ist das Ca-Salz immer weniger giftig als das Na-Salz desselben Anions. Ist aber das Anion sehr ungiftig (z. B. Cl), so daß die Versuche sich in relativ hohen Konzentrationen abspielen, so macht sich der Umstand geltend, daß Na in höheren Konzentrationen weniger schädlich ist als Ca. Der hier betonte Einfluß der Konzentration dürfte mit dem Einfluß der Salze auf die Oberflächenlamelle zusammenhängen.

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Unterscheidung kolloidaler und osmotischer Schwellung beim Muskel.

Von

R. Beutner.

(Aus der Biologischen Abteilung des Rockefeller Institute for Medical Research, New York.)

(Eingegangen am 9. Januar 1912.)

Mit 4 Figuren im Text.

Die folgenden Untersuchungen behandeln quantitativ die Quellung und Entquellung von Froschmuskeln (gastrocnemius) in Mischungen von Säuren und Salzen verschiedener Art und Zusammensetzung; die numerischen Ergebnisse sind am Schluß der Arbeit tabellarisch zusammengestellt.

Diese Arbeit wurde auf Veranlassung von Herrn Professor Jacques Loeb ausgeführt und schließt sich an die von ihm vor 15 Jahren ausgeführten Untersuchungen[1]) über die osmotischen Eigenschaften des Muskels an.

1. Damals wurde von ihm gefunden, daß in $^1/_8$ mol. NaCl-Lösung der Froschmuskel im allgemeinen für lange Zeit sein Gewicht nicht ändert; $^1/_8$ mol. NaCl ist also mit dem Muskel isosmotisch im Sinne der aus pflanzenphysiologischen Beobachtungen zuerst gewonnenen Erfahrungen. Bei Zusatz von sehr wenig Säure jedoch verhält sich die $^1/_8$ mol. NaCl-Lösung sehr verschieden, der Muskel nimmt sehr große Mengen Wasser auf. Organische Säuren wirken hierbei, wie Loeb fand, stärker, als man nach ihrem Dissoziationsgrad erwarten sollte. Muskeln, die längere Zeit in neutraler NaCl-Lösung gelegen haben, nehmen infolge von Zersetzungen eine saure Reaktion

[1]) Loeb, Arch. f. d. ges. Physiol. 69, 1; 71, 457.

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an und beginnen auch zu schwellen. Hieraus ergab sich weiter eine Erklärungsmöglichkeit für ödematische Erscheinungen: wenn es im Muskel infolge von Sauerstoffmangel zu einer Säurebildung kommt, so muß das zu einer Schwellung führen.

Der Zweck der vorliegenden Untersuchung war es zunächst, die quantitative Beziehung zwischen Salz- und Säure-Konzentration bei den Quellungserscheinungen festzustellen. Alle Säuren rufen Wasseraufnahme des Muskels hervor, um so stärker, je höher die Konzentration ist (bis zu einer gewissen oberen Grenze). Salze wirken der Schwellung entgegen, ebenfalls mit wachsender Konzentration in steigendem Maße, bei genügend hoher Konzentration ist diese antagonistische Wirkung so stark, daß der Muskel schrumpft[1]). Bestimmt man nun diejenigen Salzkonzentrationen, die bei verschiedenen Konzentrationen derselben Säure das Muskelgewicht gerade unverändert lassen, so ergibt sich zwischen Salz- und Säure-Konzentration keine Proportionalität, vielmehr nimmt, wie die folgenden Zahlen zeigen (siehe auch Tabellen unter 1), das Verhältnis mit steigender Säurekonzentration ab.

NaCl—HCl-Mischungen.

Konz. an HCl	Konz. an NaCl, die eben Quellung verhindert	$\frac{[NaCl]}{[HCl]}$
$^1/_{2000}$ normal	$^1/_8$ bis $^1/_4$ mol.	250 bis 500
$^1/_{1000}$ „	$^1/_8$ „ $^3/_{16}$ „	125 „ 187,5
$^1/_{500}$ „	$^3/_{16}$ „ $^7/_{32}$ „	93,8 „ 109,2
$^1/_{200}$ „	$^5/_{16}$ „ $^6/_{16}$ „	62,5 „ 75
$^1/_{100}$ „	$^3/_8$ „ $^7/_{16}$ „	37,5 „ 43,7

Ähnlich bei

Na_2SO_4—H_2SO_4-Mischungen. (Tabellen unter 2.)

Konz. an H_2SO_4	Konz. an Na_2SO_4, die Quellung verhind. n. 24°	$\frac{[Na_2SO_4]}{[H_2SO_4]}$
$^1/_{1000}$ normal	0,05 bis 0,06 mol.	50 bis 60
$^1/_{200}$ „	0,01 „ 0,02 „	2 „ 4
$^1/_{50}$ „	0,02 „ 0,03 „	1 „ 1,5

[1]) Diese Tatsache wurde zuerst von Loeb beobachtet (Arch. f. d. ges. Physiol. 75, 303, Dynamik d. Lebenserscheinungen, S. 77) und wurde später von M. H. Fischer verallgemeinert (Das Ödem, Dresden 1911).

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Bei H_2SO_4 findet zwischen $^n/_{1000}$ und $^n/_{200}$ sogar eine absolute Abnahme der entquellenden Salzkonzentration statt. Bei Versuchen mit Essigsäure-NaCl (nicht in Tabellen mitgeteilt) ergab sich die entquellende Salzmenge unverändert zwischen $^1/_8$ und $^3/_{16}$ mol. bei allen Säurenkonzentrationen ($^n/_{1000}$ bis $^n/_{50}$), d. h. das Verhältnis Salz : Säure nimmt auch hier stark ab.

2. Was nun die Erklärung der Quellungserscheinungen betrifft, so nahm Loeb an, daß in erster Linie osmotische Kräfte in Betracht kommen, daß aber weiterhin „die in die Gewebe eindringenden Säuren (und Alkalien) namentlich durch ihre chemische Verbindung mit den Eiweißkörpern Zustandsänderungen im Muskel schaffen, wodurch die Bedingungen der Wasserabsorption und Wasserabgabe geändert werden“ [1]). Es entsteht nun die Frage, inwieweit diese Vorgänge der Wasseraufnahme und -abgabe des Muskels durch Änderung des osmotischen Druckes und inwieweit als ein Analogon der Schwellungserscheinungen kolloidaler Proteine (wie Gelatine) erklärt werden können.

Fischer[2]) nimmt an, daß die osmotischen Erscheinungen überhaupt keine oder nur eine sehr nebensächliche Rolle bei diesen Vorgängen spielen. Die vorliegende Untersuchung zeigt, daß diese Ansicht unhaltbar ist.

Wir gehen von den Schwellungs- und Entschwellungserscheinungen des Muskels in Mischungen von Na_2SO_4- und H_2SO_4-Lösungen aus. Bestimmt man diejenige Konzentration des Na_2SO_4, die in einer beispielsweise $^n/_{200}$-H_2SO_4-Lösung aufzuwenden ist, um nach ca. 24 Stunden weder Schwellung noch Entschwellung hervorzubringen, so findet man, daß dieselbe zwischen 0,01 und 0,02 mol. Na_2SO_4 liegt. (Siehe Tabelle 2c, bei 0,01 oder weniger mol. Na_2SO_4-Schwellung, bei 0,02 oder mehr Entschwellung). Diese Na_2SO_4-Konzentration ist nun außerordentlich verschieden von derjenigen, die bei neutraler Reaktion das Muskelgewicht unverändert läßt; dies ist eine mit $^1/_8$ mol. NaCl isomolekulare Lösung, also etwa 0,1 mol. Na_2SO_4. 0,02 mol. Na_2SO_4 wirkt entschwellend, obgleich es bei neutraler Reaktion stark hypotonisch ist, aber

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[1]) Loeb, Dynam. d. Lebensersch. S. 77 und 78.

[2]) l. c.

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diese Entschwellung tritt erst nach mehreren Stunden ein, und es geht ihr eine Periode starker Wasseraufnahme voraus. In den ersten 3 Stunden und noch etwas länger verhält sich die saure Lösung ebenfalls hypotonisch, erst längere Zeit, nachdem der Muskel unerregbar geworden ist, setzt die entschwellende Wirkung ein. Der zeitliche Verlauf der Wasseraufnahme resp. -abgabe ist also durch eine Kurve der folgenden Art (Fig. 1 resp. 2) darstellbar:

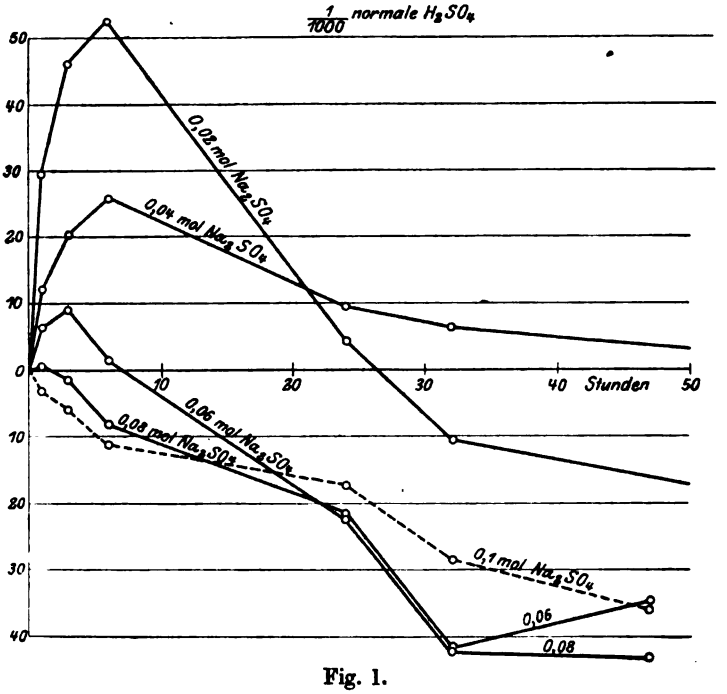


Fig. 1.

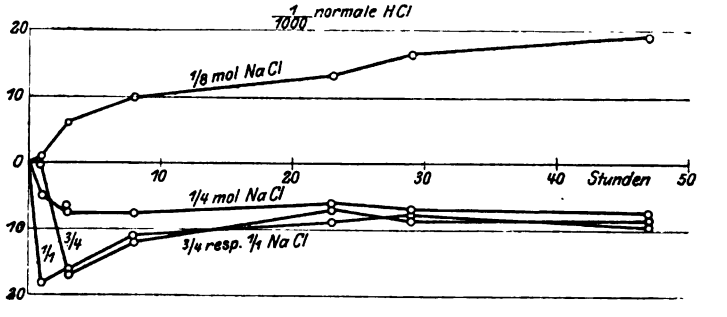


Fig. 2.

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Daß die postmortale Schwellung eine „kolloidale" Wasseraufnahme ist, ergibt sich mit erheblicher Gewißheit daraus, daß sie sich durchaus so verhält, wie man es nach dem analogen kolloidalen Zustandsänderungen anderer Proteine erwarten sollte. Diese Zustandsänderungen sind von Hardy, Pauli u. a. in einer großen Reihe von Untersuchungen beschrieben worden[1]). Es handelt sich im wesentlichen um folgende Tatsachen. Verschiedene gelöste Proteine werden durch Zusatz von Säuren (oder Basen) in ihrer Wanderungsrichtung im elektrischen Feld in gleicher Weise beeinflußt, ebenso wird ihre Viscosität erhöht, ihre Fällbarkeit durch Alkohol aufgehoben oder vermindert, und der Koagulationspunkt erhöht; bei festen Kolloiden tritt durch Säurezusatz Schwellung ein. Bei allen diesen Erscheinungen (außer der erstgenannten, elektrischen Wanderung) wirkt Salzzusatz antagonistisch, ganz wie bei der Muskelschwellung. Ferner ist es allgemein, daß HCl die spezifischen Säurewirkungen viel stärker zeigt als H_2SO_4; gerade das ist offenbar die Ursache der in dieser Arbeit behandelten Tatsache, daß eine viel kleinere Na_2SO_4-Menge die H_2SO_4-Schwellung aufheben kann, als NaCl die HCl-Schwellung.

Durch einige eigene Versuche überzeugte ich mich von der sehr verschiedenen Einwirkung der Säuren auf Eiweißlösungen; ich beobachtete die Änderung der Alkoholfällbarkeit, was sehr leicht auszuführen ist. 2 ccm 2%ige Gelatinelösung + 1 ccm $^n/_{10}$-H_2SO_4 + 10 ccm 95%iger Alkohol ergab eine starke weiße Fällung, während 2 ccm 2%ige Gelatine + 1 ccm $^n/_{10}$-HCl + 10 ccm Alkohol völlig klar blieb. Ebenso fiel der Versuch aus, wenn statt HCl HNO_3 verwendet wurde; HNO_3, das bei den Muskelschwellungsversuchen eine mittlere Stellung zwischen H_2SO_4 und HCl einnimmt, verhält sich hier mehr dem HCl ähnlich. Die Wirkung von HCl und H_2SO_4 ist aber sehr deutlich verschieden und durchaus der Einwirkung auf den Muskel analog. Sehr deutlich ist auch folgender Versuch: eine klare Mischung von 10 ccm 2%ige Albuminlösung + 2 ccm $^n/_{10}$-HCl + 10 ccm Alkohol wird durch Zusatz von 2 ccm $^n/_{10}$-H_2SO_4 sofort gefällt, Zusatz von 2 ccm $^n/_{10}$-HCl oder HNO_3 ist dagegen ohne Wirkung.

[1]) Eine Literatur-Übersicht findet man bei Handovsky, Koll. Zeitschr. 8, 183 und 267.

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Der Einwand, daß die Schwellung des noch reizbaren Muskels nicht osmotischer Natur, sondern etwa auch kolloidal sei, verdient kaum Erwähnung, aus den Tabellen ergibt sich, daß für diese Schwellung nur die Konzentration in Frage kommt.

Wichtigste Resultate der Arbeit: 1. Quellung ist beim lebenden Muskel im wesentlichen durch osmotische Vorgänge, beim toten durch kolloidale bedingt.

2. H_2SO_4 zeitigt die postmortale Schwellung weniger als HCl analog dem Verhalten gegenüber anderen Proteinen.

Herr Prof. J. Loeb gewährte mir bei Ausführung dieser Untersuchung stets in freundlichster Bereitwilligkeit seinen Rat und seine Unterstützung, wofür ich ihm sehr zu Dank verpflichtet bin.

Tabellen[1]).

1. Schwellung des Froschmuskels (Gastrocnemius) in HCl—NaCl-Mischungen.

a) $^n/_{2000}$-HCl.

	Nach 1	4	7	24	30	48 Std.
1/8 mol. NaCl	−0,4	−0,3	+1,5†	+6,7	+6,9	+5,6
1/4 „ „	−5,5	−9,3†	−9,1	−6,2	−5,6	−8,9
1/2 „ „	−9,5	−15,2†	−12,6	−6,6	−6,2	−6,2
1/1 „ „	−14,8	−16†	−9,8	−1,6	+3,2	+7,3

b) $^n/_{1000}$-HCl.

	Nach 1	3	6	23	29	47 Std.
1/8 mol. NaCl	+1†	+6,1	+9,3	+13,6	+16,3	+19,2
1/4 „ „	−5,0†	−7,9	−7,9	−5,9	−6,8	−7,7
1/2 „ „	−9,5†	−17,7	−13,0	—	—	—
3/4 „ „	[+0,1?]†	−17,2	−12,1	−7,3	−8,6	−8,1
1/1 „ „	−18,8	−17,0	−11,2	−8,5	−7,4	−9,6

c) $^n/_{500}$-HCl.

	Nach 2	4	6	24	29	48 Std.
1/8 mol. NaCl	+7,0†	+8,1	+10,6	+13,0	+14	+16,1
5/32 „ „	+1,4†	+1,5	+2,8	+8,3	+9,7	+9,7
3/16 „ „	−0,5	+0,2†	+2,5	+16	+22,4	+29,1
7/32 „ „	−3,1	−4,0†	−3,1	−2,4	−2,2	−2,9
1/4 „ „	−4,4	−6,4†	−6,0	−5,3	−5,2	−7,0
1/2 „ „	−13	−13,5	−13,2	−13,6	−14,7	—
1/1 „ „	−15,4	−11,5	−10,0	−7,4	−8,8	—

[1]) Die Zahlen bedeuten % des Gewichtes. Kreuz (†) neben einer Zahl bedeutet keine Erregbarkeit mehr vorhanden. Sternchen (*) neben einer Zahl bedeutet Erregung nur noch im allerschwächsten Maß vorhanden.

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Bemerkung: Der Versuch mit $^1/_8$ mol. NaCl, wurde dreimal, derjenige mit $^1/_4$ mol. NaCl zweimal wiederholt; da bei diesen Versuchen der beschleunigende Einfluß der Temperatur nicht berücksichtigt wurde, können Abweichungen von 10% bei starker Schwellung nach 5 und mehr Stunden vorkommen. Der Sinn der Gewichtsänderung ist indes stets der gleiche. Bei entschwellenden Muskeln betragen die Abweichungen zwischen einzelnen Versuchen 2 bis 3%.

d) $^n/_{200}$-HCl.

	Nach 2	4	6	22	28	46 Std.
$^1/_8$ mol. NaCl	+ 15,5†	+ 27	+ 31	+ 55	+ 60	+ 49
$^1/_4$ „ „	+ 2,2†	+ 4,6	+ 8,7	+ 26,5	+ 28,7	+ 11,2
					32 Std.	
$^5/_{16}$ „ „	− 2,9†	− 3,6	− 4,4	+ 0,3	+ 0,1	—
$^6/_{16}$ „ „	− 5,7†	− 11,3	− 14	− 9,3	− 7,5	—
$^7/_{16}$ „ „	− 6,7	− 12,4	− 13,5	− 10,2	− 9,3	—
$^1/_2$ „ „	− 16,6	− 18,2	− 17,3	− 17,7	− 17,1	− 17,5
$^1/_1$ „ „	− 17,2	− 16,2	− 18,2	− 24,0	− 27	− 28,7

e) $^n/_{100}$-HCl.

	Nach 1	3	6	23	48 Std.
$^1/_4$ mol. NaCl	+ 0,5†	+ 2,8	+ 5,9	+ 13,8	+ 11,2
$^5/_{16}$ „ „	− 1,7†	− 1,5	+ 0,2	+ 7,0	+ 3,1
$^3/_8$ „ „	− 2,9	− 3,8	− 4,0	+ 0,6	+ 3,3
$^7/_{16}$ „ „	− 5,9	− 10	− 10,7	− 8,2	− 7,7
$^1/_2$ „ „	− 7,6	− 14	− 13,4	− 13,2	− 10,9
$^5/_8$ „ „	− 14,7	− 24,8	− 23,6	− 25,1	− 25,0
$^3/_4$ „ „	− 10.9	− 19,6	− 20,7	− 23,9	− 26,0
$^1/_1$ „ „	− 18	− 25	− 27	− 31	− 32,6

2. Schwellung des Gastrocnemius in H_2SO_4—Na_2SO_4-Mischungen.

a) $^n/_{2000}$-H_2SO_4.

	Nach 1	3	6	24	32	47 Std.
0,06 mol. Na_2SO_4	− 8,0†	− 12,0	− 12,7	− 4,8	− 5,2	− 11,1
0,08 „ „	+ 2,9†	+ 2,9	− 5,8	− 15,3	− 21,2	− 33,6
0,1 „ „	− 3,6†	− 5,8	− 10,1	− 19,5	− 26,1	− 39,1

b) $^n/_{1000}$-H_2SO_4.

	Nach 1	3	6	24	34	47 Std.
0,02 mol. Na_2SO_4	+ 29,5*	+ 46,1†	+ 53,6	+ 4,8	− 15,5	—
0,04 „ „	+ 12,2*	+ 21,2†	− 25,7	+ 9,7	+ 7,1	—
0,05 „ „	+ 10,5*	+ 18,8†	+ 23,8	+ 7,5	+ 4,6	—
0,06 „ „	+ 6,5*	+ 8,7†	+ 0,5	− 23,3	− 43	− 35
0,08 „ „	+ 0,5*	− 1,6†	− 8,1	− 22	− 43	− 43,5
0,1 „ „	− 2,8†	− 6,0	− 11,5	− 17	− 28,5	− 37,3

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c) $^n/_{200}$-H_2SO_4.

		Nach	1	3	6	25	28	48 Std.
0,01	mol.	Na_2SO_4	+ 14,1*	+ 24†	+ 23,2	+ 13,9	+ 8,0	+ 3,0
0,02	„	„	+ 19,1†	+ 27	+ 17,6	− 7,6	− 11,9	− 8,4
0,03	„	„	+ 14,1†	+ 18,3	+ 11,9	− 1,9	− 7,6	− 7,4
		Nach	1	3	6	24	32	96 Std.
0,04	mol.	Na_2SO_4	+ 9,9	+ 12,3	+ 1,8	− 19,3	− 25,2	− 31,2
0,05	„	„	+ 7,3	+ 8,5	+ 0,1	− 23,0	− 30,5	− 31

d) $^n/_{50}$-H_2SO_4.

		Nach	1	3	6	25	28	48 Std.
0,01	mol.	Na_2SO_4	+ 23†	+ 21,9	+ 14,3	+ 17,9	+ 16,0	+ 16,1
0,02	„	„	+ 13,1†	+ 15,2	+ 10,7	+ 12	+ 6,1	+ 2,5
0,03	„	„	+ 11,3†	+ 5,1	− 2,4	− 1,3	− 5,9	− 5,2

3. Schwellung des Gastrocnemius in $NaNO_3$—HNO_3-Mischungen.

a) $^n/_{2000}$-HNO_3.

		Nach	1	3	6	23	28	48 Std.
0,05	mol.	$NaNO_3$	+ 16,8	+ 29	+ 18,2	− 18,4	− 25,5	− 42.7
0,075	„	„	+ 8,7	+ 19,1	+ 20,0	− 11,8	− 24,9	− 43,3

b) $^n/_{500}$-HNO_3.

		Nach	1	3	6	23	28	48 Std.
0,05	mol.	$NaNO_3$	+ 20,2	+ 25,7	+ 26,9	+ 15,9	+ 15,9	—
0,075	„	„	+ 16,1	+ 22,8	+ 24,3	+ 13	− 4,8	—
0,1	„	„	+ 10,7	+ 16,2	+ 13,7	− 1,5	− 9,7	− 12

c) $^n/_{100}$-HNO_3.

		Nach	1	3	6	23	28	48 Std.
0,05	mol.	$NaNO_3$	+ 19,4†	+ 39,0	+ 46,0	+ 67	+ 32,9	—
0,1	„	„	+ 12,8†	+ 17	+ 20,1	+ 14,8	+ 8,1	− 3,6
0,3	„	„	− 8,2†	− 14,4	− 22,6	− 29,1	− 35,3	− 34,3
0,5	„	„	− 17,5†	− 23,2	− 33,3	− 36,2	− 39,4	− 37

Nachtrag bei der Korrektur.

Zu meinem Bedauern habe ich bei Abfassung der vorstehenden Abhandlung eine Arbeit von Edwards B. Meigs[1]) über den gleichen Gegenstand übersehen und deshalb nicht gebührend zitiert. Meigs findet durch Versuche anderer Art als die meinen (Vergleich von frischem und mit destilliertem Wasser abgetötetem Muskel) das gleiche Resultat; daß osmotische Schwellung bei dem lebenden, kolloidale bei dem toten Muskel vorliegt, im Gegensatz zu M. H. Fischer, der diese verschiedenen Arten Quellung nicht genügend berücksichtigt.

[1]) Amer. Journ. of Physiol. 26, 191.

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Über die Abhängigkeit der Zahl der Herzschläge vom Partialdruck des Sauerstoffs.

Von

Jacques Loeb und Hardolph Wasteneys.

(Aus dem Rockefeller Institute, New York.)

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I.

Um zu einer Entscheidung darüber zu gelangen, ob die Zahl der Herzschläge durch chemische Prozesse bestimmt sei, veranlaßte Loeb im Jahre 1905 Herrn C. D. Snyder, den Temperaturkoeffizienten für die Geschwindigkeit der Pulsationen zu bestimmen. Für chemische Vorgänge dürfen wir im allgemeinen erwarten, daß eine Temperaturerhöhung von 10° ihre Geschwindigkeit mindestens ungefähr verdoppelt. Alle bisher vorliegenden Beobachtungen an den Herzen von Kaltblütern — von Yung, Snyder, Robertson, Rogers — ergaben, daß der Temperaturkoeffizient für die Zahl der Herzschläge diese Größenordnung besitzt.

Bei der Verfolgung der Frage, ob die Oxydationsvorgänge die unabhängige Variable bei den Lebenserscheinungen sind, dürfte deshalb die Bestimmung des Zusammenhangs zwischen Oxydationen und Zahl der Herzschläge in der Zeiteinheit von Bedeutung sein. Loeb hat diesen Zusammenhang schon vor 17 Jahren zum Gegenstand einer Untersuchung gemacht[1]). Als Objekt dienten Fischembryonen, bei denen sich die Herztätigkeit bequem unter dem Mikroskop beobachten läßt. Bei den Embryonen von Fundulus stellte sich heraus, daß, wenn man die Embryonen in einer Engelmannschen Gaskammer beobachtet, aus der die Luft durch einen Strom von reinem Wasser-

[1]) Arch. f. d. ges. Physiol. 62, 249, 1895.

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stoff ausgetrieben wird, die Geschwindigkeit der Herzschläge erst rasch, dann immer langsamer abnimmt, um schließlich eine Reihe von Stunden auf einem ziemlich niedrigen Niveau annähernd konstant zu bleiben. Der rasch absteigende Teil der Kurve der Zahl der Herzschläge in der Zeiteinheit bei allmählicher Sauerstoffentziehung weist unzweideutig auf eine Abhängigkeit der Zahl der Herzschläge von Oxydationen hin. Der nahezu asymptotische Verlauf der Kurve nach längerer Durchströmung ist nicht so einfach zu erklären. Man könnte daran denken, daß hydrolytische Prozesse für die Unterhaltung der Herztätigkeit in diesem Stadium des Sauerstoffmangels in Betracht kommen. Es ist ja möglich, daß ein und derselbe oder ein nahe verwandter Stoff durch Oxydation sowohl wie durch Hydrolyse entstehen könnte, und daß die Geschwindigkeit der Bildung dieses oder dieser Stoffe die Geschwindigkeit der Herzschläge bestimmt. Ist nun die Geschwindigkeit, mit der diese Stoffe durch die Oxydation gebildet werden, relativ groß, während die Geschwindigkeit der Bildung dieser oder verwandter Stoffe durch Hydrolyse relativ klein ist, so läßt sich das Verhalten der Herzen von Fundulus bei kontinuierlicher Durchströmung mit Sauerstoff verstehen. Die Kurve der Geschwindigkeit der Herzschläge bei stetig abnehmendem Partialdruck des Sauerstoffs läßt sich unter dieser Voraussetzung in zwei Teile spalten. Der erste rasch abfallende Ast entspricht der Abnahme der Oxydationsprodukte, die die Herztätigkeit bestimmen. Der zweite asymptotisch zur Abszissenachse verlaufende Teil der Kurve entspricht der Unterhaltung der Herztätigkeit durch Spaltungsprodukte. In diesem Sinne hatte Loeb seine Resultate damals auch erklärt.

Öhrwall stellte am Froschherzen Versuche über die Entstehung der sogenannten Lucianischen Gruppenbildung an und fand ebenfalls, daß bei Sauerstoffmangel die Geschwindigkeit der Herzschläge abnimmt[1]).

Wir werfen in dieser Abhandlung die folgende Frage auf: Wenn die Oxydationen oder die Masse eines oder mehrerer bestimmter Oxydationsprodukte die Geschwindigkeit der Herzschläge bedingt, so müßte es gelingen, durch passend gewählte

[1]) Skand. Arch. f. Physiol. 8, 1, 1898.

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Herabsetzung des Sauerstoffdruckes die Geschwindigkeit der Herzschläge tagelang auf einem niedrigeren Niveau zu erhalten; und zwar ebenso sicher wie das durch Herabsetzung der Temperatur möglich ist. Um solche Versuche auszuführen, muß der Vorrat von Sauerstoff relativ groß sein, so daß man bei der Ausführung dieses Versuches auf kleine Organismen angewiesen ist. Wir beschlossen, diese Versuche bei den Embryonen von Fischen, nämlich Fundulus, auszuführen.

II.

Genauer formuliert ist unser Problem wie folgt:

Die früheren Versuche von Loeb hatten ergeben, daß, wenn wir reinen Wasserstoff durch die Engelmannsche Kammer leiten, die Zahl der Herzschläge von Fundulus in der Zeiteinheit mehr und mehr abnimmt. Die Frage, die uns vornehmlich interessiert, ist die, ob die zu irgendeiner Zeit in einem solchen Versuche beobachtete Geschwindigkeit der Herzschläge der Ausdruck der um diese Zeit im Herzen (oder dem Remakschen Ganglion) herrschenden Oxydationsgeschwindigkeit ist. Wenn das der Fall wäre, so sollte folgender Versuch gelingen. Nehmen wir an, daß ein Herz zu Anfang des Versuchs mit einer Geschwindigkeit von 20 Pulsationen in 6 Sek. schlägt, und daß nach zweistündiger Durchströmung mit einem bestimmten Gemisch von Luft und Wasserstoff die Zeit auf das dreifache erhöht wird. Wir nehmen weiter an, daß um diese Zeit der Gasstrom unterbrochen werde, und daß das Herz dauernd in dem Luft-Wasserstoffgemisch bleibe und daß das Volumen des Gases, das dem Herzen zur Verfügung steht, hinreichend groß ist. In dem Falle sollte das Herz tagelang bei gleicher Temperatur mit der Geschwindigkeit schlagen, die beobachtet wurde, als die Durchströmung unterbrochen wurde. Wenn das der Fall wäre, so könnte man daran denken, daß die zu irgendeiner Zeit bei Wasserstoffdurchströmung beobachtete Geschwindigkeit der Herzschläge der direkte Ausdruck der Oxydationsgeschwindigkeit im Ganglion oder Herzen ist.

Diesen Versuch haben wir ausgeführt. An ein Gasometer, das ein Gemisch von 10 Volumprozent Luft und 90 Volumprozent Wasserstoff enthielt, wurden vier Glaszylinder, jeder von ca. 200 ccm Volumen, angeschlossen (von denen jeder sechs

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Fundulusembryonen in 20 ccm Seewasser erhielt), und außerdem eine Engelmannsche Gaskammer, in der die Herzschläge bei der Durchströmung gezählt werden konnten. Jeder Zylinder hatte einen luftdicht eingeschliffenen Glasdeckel, in den zwei Glasröhren eingeschmolzen waren, eine zum Einführen, die andere zum Ausführen der Gase. Jede Röhre war mit einem luftdicht schließenden Einweghahn versehen. Wenn die Durchströmung beendet war, so konnte man durch Schließen der Hähne jeden Zylinder luftdicht abschließen. Es wurde nun ein kräftiger Strom des Gemisches von 10 % Luft und 90 % Wasserstoff 3 Stunden und 15 Minuten lang durch die Zylinder getrieben und dann die einzelnen Flaschen durch Schließen der Hähne isoliert. Die Herzen der beobachteten vier Embryonen in der Gaskammer brauchten zu Beginn des Versuches für 20 Pulsationen 5 Sekunden (Temperatur 24,7°). Eine Stunde später brauchten sie für 20 Pulsationen 13″, 16″, 17½″ und 16″ bei 23°, und 3 Stunden 15 Minuten nach Beginn der Durchströmung 13″, 20″, 13″ und 18″ bei 22°. Dann wurde, wie gesagt, jeder Zylinder isoliert.

Nach 24 Stunden wurde der erste Zylinder geöffnet, 5 Embryonen so rasch wie möglich in ein Uhrschälchen gebracht und die Geschwindigkeit der Herzschläge durch Zählen festgestellt. Es ergaben sich folgende Zeiten für 20 Pulsationen:

16½″ 20″ 17″ 16″ 16″ Temperatur 22°.

Die Zahl der Herzschläge war also unverändert geblieben.

An der Luft erreichte die Herztätigkeit alsbald wieder ihre normale Geschwindigkeit. Nach einer Stunde waren die Zeiten für 20 Pulsationen:

10½″ 10¼″ 10″ 10¼″ 10″ Temperatur 22°

und einige Stunden später:

7″ 8″ 8″ 7¾″ 8″ Temperatur 24,8°.

Am nächsten Tage, also 48 Stunden nach Beginn des Versuches, wurde der zweite Zylinder geöffnet und die folgenden Zeiten wurden für 20 Pulsationen gefunden:

20″ 19″ 16″ 16″ Temperatur 22,7°.

In Berührung mit Luft ging die Zeit im Laufe einiger Stunden zurück, nämlich:

9″ 8″ 9″ 9″ Temperatur 23°.

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Der dritte Zylinder wurde 2 Tage später geöffnet, also am vierten Tage nach Beginn des Versuches. Infolge eines Zufalles dauerte es einige Minuten, ehe die Zahl der Herzschläge festgestellt wurde. Für 20 Pulsationen wurde gefunden:

14″ 14″ 14½″ 12½″ 13″ Temperatur 23°.

Der Umstand, daß die Zeiten etwas kleiner ausfielen als in den anderen Fällen, dürfte vermutlich darauf zurückzuführen sein, daß die Eier einige Minuten mit der Luft in Berührung waren, ehe die Zählung der Herzschläge stattfand.

Als der vierte Zylinder am folgenden Tage geöffnet wurde, stellte es sich heraus, daß die Herzen der meisten Embryonen stillstanden. An der Luft aber erholten sie sich bald und fingen wieder an zu schlagen.

Eine Wiederholung derselben Versuche gab ganz ähnliche Resultate. Das Gasgemisch war wieder 10 Volumprozent Luft und 90 Volumprozent Wasserstoff (durch Analyse kontroliert).

Der nach 24 Stunden geöffnete Zylinder mit 9 Embryonen gab folgende Zeiten für 20 Pulsationen:

15″ 19″ 16″ 13″ 14″ 15″ 13″ 12″ 13″ Temperatur 24,9°.

Der nach 2 Tagen geöffnete Zylinder ergab:

12,6″ 21,2″ 14,2″ 18″ 20″ 14,4″ 12,5″ 15,6″ 11,4″ 20,8″ 12,5″ Temperatur 23,8°.

Der nach 3 Tagen geöffnete Zylinder ergab:

14″ 20″ 12″ 20″ 16,4″ 15,4″ Temperatur 24,2°,

Bei dieser Temperatur war die normale Zeit etwa 7″.

Diese Versuche scheinen die Ansicht zu stützen, daß einem bestimmten niedrigen Sauerstoffdruck auch eine bestimmte und für eine Reihe von Tagen bei derselben Temperatur konstante erniedrigte Geschwindigkeit der Pulsationen des Herzens entspricht.

III.

Eine zweite Versuchsreihe wurde mit Embryonen ausgeführt, die mit verschiedenen Mengen NaCN vergiftet waren. Die Methode bestand darin, daß wir ein, zwei oder mehr Tropfen einer $^1/_{10}$ %igen Lösung von NaCN zu je 50 ccm Seewasser zusetzten und ein oder mehrere Eier mit Fundulusembryonen hineinbrachten. Das Seewasser mit den Embryonen befand sich in Erlenmeyerschen Flaschen, die mit Gummi-

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stöpsel luftdicht verschlossen waren. Jede Flasche hatte ein Volumen von etwa 200 ccm. Der Sauerstoffinhalt war also mehr als ausreichend. Von Zeit zu Zeit, gewöhnlich einmal jeden Tag, wurden die Eier herausgenommen und mit einer Stoppuhr die Zeit bestimmt, die für 20 Pulsationen erforderlich ist. Dann wurden die Eier wieder in die Flasche zurückgebracht. Hierbei wurde durch Verdunstung von HCN die Konzentration von NaCN etwas herabgesetzt.

Zur Theorie der Versuche sei folgendes bemerkt. Wir nehmen an, daß die Geschwindigkeit der Oxydationen im Embryo u. a. der Masse der Peroxyde (Oxydationsfermente) proportional ist; und daß der Zusatz von NaCN die Geschwindigkeit der Oxydationen dadurch verringert, daß ein Teil der Peroxydmoleküle sich mit NaCN oder HCN verbindet, wodurch dieselben von der Teilnahme an der Oxydation ausgeschlossen werden.

Wir wollen nun einige Versuchsreihen mitteilen.

Die erste Reihe gibt Versuche mit je 4 Embryonen in einer Lösung und die angegebenen Zahlen sind das Mittel aus den vier Beobachtungen. Zu je 50 ccm Seewasser waren 0, 1, 2, 3, 4, 6, 9, 12, 24 Tropfen $^1/_{10}{}^0/_0$ KCN zugesetzt worden. Zu Beginn des Versuches waren die Embryonen 6 Tage alt.

Tabelle I.

Nach	Sekunden erforderlich für 20 Herzschläge in 50 ccm Seewasser + Tropfen $^1/_{10}{}^0/_0$ NaCN									Temperatur in Grad
	0	1	2	3	4	6	9	12	24	
2 Stunden	7	7 1/2	8 1/2	9	10	11	15	17	24	26,3
1 Tage	9	10 1/2	12 1/2	16	18	19	27	34	41	23,5
2 Tagen	8	11	16	20	23	25	30	36	60	23
3 „	9	11	15	20	20 1/2	28	32	40	40	23,6
4 „	8	10 1/2	17	20	21	24	35	40	40	23,3
5 „	7 1/2	9 1/2	18	21		22	26	34	40	?
6 „	7 1/2	11	18	24	42	20	68	32	42	22
7 „		8 1/2	15	25		31	64	36	tot	23,5
8 „		9	14	33	19	tot	tot	tot		

Nach 2 Stunden hatte sich das Gleichgewicht zwischen der NaCN-Lösung und den durch NaCN beeinflußten Substanzen im Ei noch nicht hergestellt; nach 24 Stunden ist das aber der Fall. Die Kontrollfische — mit 0 NaCN — schlüpften am 6. Tage aus, die Embryonen in den Gefäßen mit NaCN waren am Ausschlüpfen verhindert.

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Betrachten wir die Geschwindigkeit der Herzschläge in den Gefäßen mit 1, 2 und 3 Tropfen NaCN, so sehen wir, daß jeder Konzentration von NaCN eine nahezu konstante Geschwindigkeit der Herzschläge während den 8 Tagen der Beobachtung zukommt (unter Berücksichtigung der Temperaturunterschiede), und daß diese Geschwindigkeit um so geringer ist, je höher die Konzentration des NaCN.

Nimmt man das Mittel, so verhalten sich die Geschwindigkeiten der Herzschläge in 0, 1, 2 und 3 Tropfen wie

8,2:10,1:15,7:22,3.

Der Zusatz von 1 Tropfen NaCN setzt die Geschwindigkeit nur um 25% herab, während das Hinzufügen von 2 Tropfen die Geschwindigkeit um 25% gegen die mit 1 Tropfen herabsetzt; und die gleiche Differenz wird zwischen 2 und 3 Tropfen beobachtet. Zusatz von mehr NaCN bewirkt oft Störungen oder Stillstand in der Herztätigkeit, die das Resultat etwas unregelmäßig machen.

Die nächste Tabelle II enthält Versuche, in denen nur je ein Embryo in eine Lösung gebracht wurde; die Embryonen waren 7 Tage alt.

Tabelle II.

Nach Tagen	Sekunden erforderlich für 20 Herzschläge in 50 ccm Seewasser + ccm $^1/_{100}$% NaCN											Temperatur in Grad
	Kontrolle	0,5	1,0	1,5	2,0	2,5	3,0	3,5	4,0	4,5	5,0	
1	15	19½	20	20½	23	34	44	29	40	38	39	18,5
2	14	20	21	27½	22	35	52	30	40	46	54	18,8
4	11	19	20	20	25	33	39	38	43	45	53½	20
6	9	17	20	19	20	32	35	40	43	41½	46	21,5
8	10¾	18	26	24½	34	44	46	72	52	68	?	17,6
10	7½	11	18	22	21	38	44	+	40	41	37	23,6
12	?	13	18	20	28	+	?	+	48	58	54	19,8

Macht man die Korrekturen für die Temperaturen, so bemerkt man, daß in jeder der vertikalen Reihe die Zahl der Herzschläge ziemlich konstant bleibt. Durch Zusatz von 0,5 ccm $^1/_{100}$% NaCN zu 50 ccm Seewasser wird die Geschwindigkeit der Herzschläge um 30 bis 60% verringert. + bedeutet, daß das Herz still stand, ? bedeutet, daß es nicht gelang, die Herzschläge zu zählen.

Wir haben noch vier weitere Versuchsreihen derselben Art mit demselben Resultat angestellt.

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Aus diesen Versuchen geht hervor, daß es gelingt, durch bestimmte kleine Dosen von NaCN bei den Embryonen von Fundulus die Geschwindigkeit der Herztätigkeit für eine Reihe von Tagen auf ein relativ konstantes niedrigeres Niveau herunterzudrücken. Dieses Resultat würde man erwarten dürfen, wenn die Geschwindigkeit der Herzschläge eine direkte Funktion der Geschwindigkeit der Oxydationsprozesse wäre.

IV.

Die folgenden Versuche über die Abnahme der Herztätigkeit bei Durchströmung der Gaskammer mit bestimmten Gemischen von Luft und Wasserstoff seien nur der Vollständigkeit halber mitgeteilt. Sie können dem Gesagten nichts Neues zufügen. Wenn die Geschwindigkeit der Durchströmung in allen Fällen genau die gleiche wäre, so ließen sich solche Versuche vielleicht verwerten, um eine Beziehung zwischen Sauerstoffdruck und Geschwindigkeit der Herzschläge zu gewinnen. Obwohl wir dieses Ziel im Auge hatten, so ist die praktische Durchführung doch zu schwierig. Wir legen deshalb den Versuchen keine große Wichtigkeit bei und teilen sie nur mit, weil der eine oder andere Leser vielleicht den Wunsch haben dürfte zu wissen, wie derartige Versuche verlaufen.

Wir teilen zunächst einen Versuch mit reinem Wasserstoffgas mit. Vier Embryonen waren im Apparat. Der Verlauf ist in Tabelle III dargestellt.

Tabelle III.

Nach	Sekunden erforderlich für 20 Herzschläge				Temperatur in Grad
0′	8	8 1/2	8 3/4	8	22,8
3′	8	8	8	8	22,7
9′	9	9 1/2	8 1/2	8	23,2
16′	14	13 1/4	12	10	23,2
20′	15 1/2	14	12	12	
34′	21	20	16	16 1/4	23,5
43′	25	24	19	20	23,6
58′	28	28	24	24	23,7
68′	30	35	28	28	23,7
78′	32	36	28	30	23,7
88′	34	40	32	33	23,3
153′	39	55	44	46	22,7
213′	39	56	53	44	22,7
16h 3′	35	40	35	tot	
20h 16′	29	tot	tot		24,2
26h	tot				

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Man kann also sagen, daß eine etwa 2stündige kräftige Durchströmung der Engelmannschen Gaskammer mit H (ohne Luft) die Zeit für 20 Herzschläge etwa auf das 5fache (von 8″ auf ungefähr 40″) erhöht.

Im nächsten Versuch wurde die Gaskammer mit einer Mischung von 25 Volumprozent Luft und 75 Volumprozent Wasserstoff durchströmt. Drei Embryonen wurden verwendet, aber anfangs lag der eine Embryo ungünstig und seine Herzschläge konnten nicht gezählt werden. Unmittelbar vor Beginn der Durchströmung waren für 20 Herzschläge $8^1/_2$″ und $8^1/_2$″ erforderlich, Temperatur 23°.

Tabelle IV.

Nach	Sekunden erforderlich für 20 Herzschläge			Temperatur in Grad
0′	$8^1/_2$	$8^1/_2$		23
1h 22′	11	12		23,5
3h 57′	10	11	12	24,2
6h 02′	10	12	12	24,8
8h 02′			12	23,3

Durchströmt man also die Kammer mit einem Gemisch, das aus $^1/_4$ Luft und $^3/_4$ Wasserstoff besteht, so wird dadurch die Geschwindigkeit der Herztätigkeit um etwas weniger als 50% verringert.

Tabelle V.

80% H, 20% Luft.

Nach	Sekunden erforderlich für 20 Herzschläge			Temperatur in Grad
0′	6	$6^1/_4$	$6^1/_4$	26,5
15′	$6^1/_4$	7	$7^1/_2$	26,5
60′	$8^1/_2$	$9^1/_2$	$10^1/_2$	26,5
100′	$9^1/_2$	9	$13^1/_2$	26,6
2h 50′	10	9	18	26,6
3h 45′	9	$12^1/_4$	$18^3/_4$	26
4h 47′	10	$10^1/_4$	18	24,3

Der Leser wird bemerken, daß das dritte Herz viel langsamer schlug als die beiden anderen, es brauchte 18″ statt 10″ für 20 Herzschläge. Der betreffende Embryo mit zu langsamem Puls lag in der Mitte des Tropfens, während die beiden anderen an der Peripherie lagen und daher den Sauerstoff dem in dem Zentrum liegenden Embryo wegnahmen. Durch leichtes Neigen

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der Kammer wurde dieser dritte Embryo an die Peripherie gebracht und nun stieg die Geschwindigkeit seiner Herzschläge sofort auf die normale Höhe, wie folgende Messung zeigt.

				Temperatur
Nach $5^h 20'$	10″	$11^1/_2$″	$10^1/_2$″	24,7°
„ $7^h 30'$	11″	13″	11″	

Wir dürfen also sagen, daß Durchströmung der Gaskammer mit einem Gemisch von 20 Volumprozent Luft und 80 Volumprozent Wasserstoff in etwa 2 Stunden die Geschwindigkeit der Herztätigkeit um etwa 50% herabsetzt, und daß die Geschwindigkeit dann stundenlang auf dieser Höhe bleiben kann.

Der folgende Versuch gibt die Resultate bei der Durchströmung mit einem Gemisch von $4^1/_2$ Volumen H und 1 Volumen Luft. Es wurden sechs Embryonen benutzt. Unmittelbar vor Beginn der Durchströmung erforderten 20 Herzschläge $7^1/_4$, $7^1/_2$, 6 und 7 Sekunden bei 25°. Den weiteren Verlauf gibt die Tabelle VI.

Tabelle VI.

82 Vol. H, 18 Vol. Luft.

Nach	Sekunden erforderlich für 20 Herzschläge						Temperatur in Grad
30′	$11^1/_4$	$10^3/_4$	$9^3/_4$	$18^1/_4$	17	$9^3/_4$	25
$1^h 05'$		$13^1/_2$	$11^1/_2$	32	14	$11^3/_4$	25
$1^h 55'$		15	$11^1/_4$	42	11	$10^1/_2$	25,3
$2^h 25'$	14	12	11	38	$11^1/_4$	11	25,8
$3^h 40'$	13	$12^1/_2$	11	40	26	12	25,5
$6^h 05'$	$12^1/_2$	$12^1/_2$	11	44	32	$10^3/_4$	24,5
$10^h 10'$	14	14	12	40	30	11	25

Um diese Zeit wurde Luft zugelassen. 2 Minuten später ergab sich das folgende Resultat:

							Temperatur
$10^h 12'$	11″	$13^1/_2$″	$10^1/_2$″	$10^3/_4$″	$11^1/_4$″	10″	25°

und 12 Minuten später:

$7^3/_4$″	9″	8″	$8^1/_4$″	9″	$8^1/_2$″	25,2°

Man sieht, wie rasch sich die Herzen bei Erneuerung der Luftzufuhr erholen. Woher kommt es nun, daß so große Verschiedenheiten in der Zahl der Herzschläge bei demselben

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Sauerstoffdruck vorkommen, wie sie in diesem Versuch beobachtet werden? In diesem Falle können wir wieder eine bestimmte Antwort auf diese Frage geben. Die beiden Embryonen, deren Herzen 40″ resp. 30″ für 20 Herzschläge brauchten, lagen wieder im Zentrum des Tropfens, erhielten also weniger Sauerstoff als die anderen. Wir dürfen deshalb diese beiden Herzen nicht für das Resultat verwerten. Wir sehen daher, daß bei dem Sauerstoffdruck, der in diesem Versuche herrschte, die Geschwindigkeit der Herzschläge von 6″ bis $7^1/_2$″ auf 11″ bis 14″ pro 20 Contractionen herunterging, also auf nicht ganz die Hälfte.

Tabelle VII.

85 Volumprozent H, 15 Volumprozent Luft.

Nach	Sekunden erforderlich für 20 Herzschläge		Temperatur in Grad
0′	$7^3/_4$	$7^1/_4$	24,3
37′	20	13	25
55′	14	22	25,3
1h55′	16	28	26
2h35′	16	31	25,6
4h45′	17	34	25

Es war wieder das in der Mitte des Tropfens liegende Herz, das langsamer schlug, und das wir daher für das Resultat nicht benutzen dürfen. Ein Gemisch von 15 Volumprozent Luft und 85 Volumprozent Wasserstoff setzt also in 2 Stunden die Geschwindigkeit der Herzschläge auf etwas weniger als die Hälfte herunter.

Tabelle VIII.

90 Volumprozent H, 10 Volumprozent Luft.

Nach	Sekunden erforderlich für 20 Herzschläge				Temperatur
0′	$8^1/_2$	$7^1/_4$	8	8	24,0
28′	14	15	$11^1/_2$	12	24,3
50′	23	20	16	19	24,0
1h28′	32	30	22	26	24,8
2h03′	40	32	28	26	25,0
3h33′	45	38	32	25	
5h53′	40	38	32	25	26,5
9h03′	36	40	31	$26^1/_2$	25,0

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Mit diesem Versuche stimmt aber der folgende Versuch mit noch niedrigerem Sauerstoffdruck nicht überein.

Tabelle IX.

95 Volumprozent H, 5 Volumprozent Luft.

Nach	Sekunden erforderlich für 20 Herzschläge				Temperatur in Grad
4′	8	8 3/4	10	9	22,8
23′	10 1/4	12	9 3/4	10 1/2	22,7
38′	13	12	15 1/2	13 1/2	22,8
1h 04′	18	19	22 1/2	16	22,7
2h 00′	26	19	44	19	22,9
4h 03′	28	19	56	20	22,5
8h 44′	24	19 1/2	50	18	22,0

Wir haben also hier eine ähnliche Wirkung wie in Tabelle VII mit 15% Luft und 85% H. Ob der Versuch in Tabelle VIII aus der Reihe fällt oder ob der Versuch in Tabelle IX abnorm ist, müssen wir unentschieden lassen. Auch mechanische Hindernisse, die der Diffusion des Sauerstoffs in den Tropfen in der Engelmannschen Gaskammer im Wege stehen, kommen bei diesen Versuchen in Betracht.

V.

Als Anhang teilen wir die folgenden Versuche mit, deren Deutung uns Schwierigkeit bereitet, weil die partiellen Sauerstoffdrucke in diesen Versuchen relativ hoch waren. Wir kommen auf die mögliche Erklärung des Zwiespalts zwischen diesen und den anderen Versuchen am Ende dieses Abschnittes zurück.

Wir stellten eine ausgedehnte Reihe von Versuchen in der Weise an, daß wir durch bestimmte kleine Mengen von alkalischem Pyrogallol einen Teil des Sauerstoffs in den Beobachtungsgefäßen absorbierten. Wir benutzten zu dem Zwecke Glasgefäße, die von Gabritschewski für Tetanuskulturen eingeführt wurden. Dieselben bestehen aus zwei flachen Schalen, die mit einem breiten, geschliffenen Rand luftdicht aufeinander passen. Die untere Schale hat eine Rinne für die Pyrogallollösung und die letztere wird durch ein kleines Loch eingeführt. Durch seitliche Verschiebung der oberen Schale wird die Kom-

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munikation des Pyrogallols mit der äußeren Luft abgeschlossen. In die Mitte dieser zwei Schalen brachten wir ein kleines Uhrschälchen, das die Eier enthielt. Dann wurde die obere Schale so aufgesetzt, daß die Löcher in beiden kommunizierten, und es wurde eine bestimmte Menge 12%iger KHO mit einer Pipette einlaufen gelassen. Dann wurde eine bestimmte Menge einer 5%igen Pyrogallollösung eingeführt und sofort die Schale gedreht, so daß das Pyrogallol keinen Sauerstoff von außen absorbieren konnte. Die Pyrogallollösung wurde immer dem fünffachem Volumen der KOH-Lösung zugesetzt.

Wir bestimmten nun empirisch diejenige Menge der Mischung, die dauernd die Zahl der Herzschläge in der Zeiteinheit auf einem niedrigen Niveau hielt, und fanden, daß das bei Zusatz von 0,3 bis 0,6 oder 0,8 ccm Pyrogallol, je nach der Kapazität der einzelnen Schalen, der Fall war. Wurde zu viel Pyrogallol zugesetzt, so daß zu viel Sauerstoff in der Schale absorbiert wurde, so verlief alles so wie in den Versuchen, in denen die Luft vollständig durch Wasserstoff verdrängt wurde.

Da Wasser stets aus dem Uhrschälchen, das die Eier enthielt, in die Rinne mit Kalilauge verdampfte, so brachten wir stark verdünntes Seewasser oder destilliertes Wasser in das Uhrschälchen. Die Eier von Fundulus entwickeln sich in destilliertem Wasser ebenso gut wie in Seewasser.

Bei diesen Versuchen tritt ein Übelstand ein, der darin besteht, daß erstens die einzelnen Eier im Uhrschälchen sich gegenseitig den Sauerstoff streitig machen, und zweitens, daß der Sauerstoff nur durch eine sehr enge Spalte von nahezu mikroskopischer Dimension in das Wasser diffundiert, das die Eier enthält, wodurch ebenfalls die Sauerstoffversorgung der Eier verzögert ist. Wir kommen auf diesen Umstand am Ende des Abschnittes noch zu sprechen. Vielleicht kommen auch individuelle Schwankungen im allgemeinen Zustand der Embryonen in Betracht, die das Resultat beeinflussen.

In dem in der folgenden Tabelle I dargestellten Versuch waren vier Herzen in derselben Gabritschewskischen Schale. Der Inhalt der Schale betrug 50,9 ccm; 0,5%iges Pyrogallol und 2,5 ccm 12% KHO waren zugesetzt. Die in derselben vertikalen Reihe stehenden Zahlen gehören nicht demselben Herzen an, da es nicht möglich war, jeden Tag dieselben Herzen

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zu identifizieren. Wir geben, wie schon erwähnt, in dieser Abhandlung nicht wie üblich die Zahl der Herzschläge in einer Minute, sondern die Zahl der Sekunden, die für 20 Herzschläge erforderlich sind[1]). Es wurden stets 20 Pulsationen gezählt und mit einer Stoppuhr die Zeit gemessen.

Tabelle X.

0,5 ccm Pyrogallol.

Nach Tagen	Sekunden erforderlich für 20 Pulsationen				Temperatur in Grad
1	11	14	16 1/2	10 3/4	24,2
2	14	20 1/2	18	12	22
3	12 1/2	16 1/2	11 3/4	12	23,5
4	17	60	18	15	22,2
5	14	52	23 1/2	16	23
6	17	68	tot	tot	
7	22	tot			22
8	36				20,8
9	18				21,5
10	15				24
41	35				20,2

Man sieht, daß die Zahlen erheblich schwanken; wir werden aber kaum irre gehen, wenn wir die minimalen Zahlen als die maßgebenden betrachten, und die höheren Zahlen als zu hoch ansehen infolge eines der vorhin erwähnten Nebenumstände.

Bei den Temperaturen, die in diesem Versuche herrschten, schwankte bei normaler Sauerstoffversorgung die Geschwindigkeit der Herzschläge zwischen 6″ und 9″ pro 20 Pulsationen; bei der hier herrschenden Sauerstoffversorgung aber schwankten die Zeiten zwischen 11″ und 18″. Die Geschwindigkeit war um etwa 100% verringert.

Wir vermeiden nun eine Quelle der Störung (nämlich die Störung der Sauerstoffversorgung, die ein Ei erfährt, wenn die benachbarten Eier ihm den Sauerstoff streitig machen), wenn wir nur ein Ei in jedes Kulturgefäß bringen. Wir wollen in der folgenden Tabelle die Beobachtung mitteilen, die gleichzeitig an 8 Embryonen angestellt wurde, die in 8 Schalen (mit steigendem Gehalt an Pyrogallol) verteilt waren.

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[1]) Das läßt den Zusammenhang der Geschwindigkeit der Herzschläge mit der Geschwindigkeit der chemischen Reaktionen besser erkennen.

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Tabelle XI.

Nach	Sekunden erforderlich für 20 Herzschläge in ccm 5%iges Pyrogallol								Temperatur in Grad
	0,1	0,2	0,3	0,4	0,5	0,6	0,65	0,7	
0h	9	8	9	7 1/2	—	—	—	—	24,5
2h 15′	9 1/2	8	10 3/4	9 2/4	14	56	48	60	23,6
1 Tag	9	7	13 1/2	10 1/4	27	50	58	58	24,2
2 Tagen	9 1/2	7	15	12	14	60	tot	54	24,2
3 „	9 1/2	7	15 3/4	13	14	tot		tot	24,1
4 „	9	7 1/4	17	?	18				24,0
6 „	10	10	13 1/2	Gruppen	20				23,6
7 „	9	7	12	16	20				25,5
8 „	7	6	11 1/2	20 1/2	tot				26,0
9 „	ausgeschlüpft	6	10	17					27,6
10 „		6 3/4	10 1/2	24					25,3
11 „		10	13 1/4	tot					21,8

Versuch abgebrochen, weil die Eier beinahe trocken waren.

Nehmen wir das Mittel aus diesen Zahlen, so erhalten wir folgende Werte für die relativen Geschwindigkeiten der Herzschläge in jeder einzelnen Schale:

I.	0	ccm Pyrogallol	9
	0,2	„ „	7,5
II.	0,3	„ „	13,0
	0,4	„ „	15,5
	0,5	„ „	18,0
III.	0,6	„ „	55
	0,65	„ „	53
	0,7	„ „	57

Hier sind deutlich drei Gruppen erkennbar. Die erste Gruppe besteht aus den beiden ersten Reihen mit 0,1 und 0,2 ccm Pyrogallol. Wir dürfen die hier beobachtete Geschwindigkeit der Pulsationen als normal bezeichnen; die geringe in diesen Gefäßen herrschende Verminderung des Partialdruckes des Sauerstoffs hatte keinen Einfluß auf die Herztätigkeit. In Gefäßen mit so wenig Pyrogallol ging auch die Entwicklung der Embryonen weiter, und dieselben schlüpften meist aus, während das in den Gefäßen mit mehr Pyrogallol nicht der Fall war. Die jungen Embryonen lebten hier lange, aber schlüpften nicht aus.

Die zweite Gruppe umfaßt die Versuche mit 0,3, 0,4 und 0,5 ccm Pyrogallol. Hier findet eine allmähliche, aber stetige

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Zunahme der für 20 Pulsationen erforderlichen Zeit statt, die bei 0,5 ccm Pyrogallol etwas über 100% erreicht.

Die dritte Gruppe umfaßt die Versuche mit mehr als 0,5 ccm Pyrogallol. Diese Gruppe ist durch einen scharfen Sprung von den voraufgehenden Versuchen getrennt. Die Geschwindigkeit der Herzschläge fällt auf ein Achtel und die Tiere sterben bald.

Wir wollen einen zweiten Versuch dieser Art mitteilen, in dem die Tatsache der Existenz dieses Sprunges ebenfalls klar zum Ausdruck kommt.

Tabelle XII.

Nach	Sekunden, erforderlich für 20 Herzschläge in ccm Pyrogallol						Temperatur in Grad
	0,3	0,4	0,5	0,6	0,7	0,8	
8 Stunden	12 1/4	12	11	14 1/4	44	54	21,2
1 Tag	13 1/2	17 1/2	15	20	68	92	20,0
3 Tagen	11 1/2	18	16	23 1/4	tot	tot	21,7
5 „	9	16 1/2	14	20			22,5
7 „	7 1/2	14	14	18			24,5
8 „	8	14	14	40			24,4
9 „	7 1/4	14	16 1/2	tot			24,8
10 „	7 1/4	13	16				24,0
11 „	7	13 1/2	tot				
13 „	7	18					23,5
18 „	ausgeschlüpft	20					21,8

Wir haben auch hier wieder die drei Gruppen. Die erste normale umfaßt nur den ersten Embryo mit 0,3 ccm Pyrogallol. In den ersten Tagen war seine Herztätigkeit etwas verzögert, dann wurde sie normal. Die in den erten Tagen bemerkbare Verlangsamung dürfte möglicherweise einer zufälligen mechanischen Hemmung der Sauerstoffversorgung infolge ungünstiger Lage des Embryos zuzuschreiben sein. Vom fünften Tage an ist der Embryo normal, was sich auch darin zeigt, daß derselbe ausschlüpft. Die zweite Gruppe umfaßt die Versuche mit 0,4, 0,5 und 0,6 ccm Pyrogallol. Hier haben wir wieder dieselbe mäßige Verlangsamung der Zahl der Herzschläge bis auf etwa die Hälfte der normalen Geschwindigkeit. Dann aber erfolgt wieder ein Sprung auf über 40″ für 20 Pulsationen, wenn wir über 0,7 ccm Pyrogallol hinausgehen.

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Da in diesen Versuchen die gebildete CO_2 von dem KHO absorbiert wurde, so kann der Sprung nicht auf eine Kohlensäurewirkung bezogen werden.

Wir kommen deshalb auf Grund dieser Versuche, die wir sehr oft wiederholten, zu dem Schluß, daß eine mäßige Herabsetzung des Partialdruckes des Sauerstoffs die Geschwindigkeit der Herzschläge um etwa 100% oder noch mehr verringert und daß das Herz mehr als eine Woche lang mit dieser verringerten Geschwindigkeit weiterschlagen kann. Sobald aber der Partialdruck unter eine bestimmte Grenze sinkt, findet eine sehr plötzliche Abnahme der Geschwindigkeit auf ein Sechstel bis ein Achtel statt, die zum raschen Tode des Embryos führt. Was den Grund für diesen Sprung bildet, können wir nicht angeben. Es wäre, wie schon erwähnt, denkbar, daß unterhalb eines kritischen minimalen Wertes des Sauerstoffdruckes die Oxydationsgeschwindigkeit im Herzen so sehr sinkt, daß die Herztätigkeit nur durch Stoffe aufrecht gehalten wird, die durch Hydrolyse entstehen.

Wir müssen nun auf eine Schwierigkeit hinweisen, die entsteht, wenn wir die Herabsetzung der Zahl der Herzschläge in diesen Pyrogallolversuchen direkt auf die Partialdrucke des Sauerstoffs zu beziehen versuchen, die in den betreffenden Gefäßen herrschten. Diese Partialdrucke waren nämlich relativ hoch im Vergleich mit den Partialdrucken, die in den früher erwähnten Versuchen benutzt wurden. Wir vermuten, daß der Partialdruck des Sauerstoffs in dem kleinen Uhrschälchen, in dem die Eier sich befanden, stets niedriger war als in der umgebenden Luft, weil, wie schon erwähnt, die Luft nur an einer mikroskopischen Spalte mit dem Wasser im Uhrschälchen in Berührung kam, wodurch die Geschwindigkeit der Sauerstoffzufuhr in dem Wasser verzögert wurde. Da nun die Eier fortwährend etwas Sauerstoff verbrauchten, so vermuten wir, daß das Wasser des Uhrschälchens immer nur unvollständig für den Partialdruck des Sauerstoffs gesättigt war, der im übrigen Gefäß herrschte. In dieser Schlußfolgerung werden wir bestärkt durch die Erfahrung über den Einfluß, den die Lage des Eies im Uhrschälchen in diesen Versuchen hatte. In den Versuchen des II. Abschnittes fiel diese Fehlerquelle fort, da hier das Wasser, in dem die Eier sich befanden, auf einer großen Oberfläche mit dem Gasgemisch in Berührung war.

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VI. Diskussion der Resultate.

Die Bedeutung des Sauerstoffs für die Herztätigkeit hat man offenbar bisher in der Physiologie für gering angesehen, was äußerlich schon daraus hervorgeht, daß dieses ganze Gebiet in dem umfangreichen Nagelschen Handbuch mit drei Sätzen erledigt wird, die hier zitiert werden mögen: „In bezug auf sein O-Bedürfnis verhält sich das Herz wie der Skelettmuskel. Auch ohne O-Zufuhr kann es noch eine Zeitlang weiter arbeiten, aber schließlich nimmt die Höhe der Contractionen enorm ab ... Am ausgeschnittenen Säugetierherzen kann der für kräftiges Schlagen nötige Bedarf an O auf lange Zeit auch ohne Anwesenheit von Hämoglobin gedeckt werden, wenn man die Durchströmungsflüssigkeit mit O unter Atmosphärendruck sättigt." Diesen Tatsachen gegenüber weisen die hier mitgeteilten Tatsachen auf die Möglichkeit hin, daß die Geschwindigkeit der Zahl der Contraktionen des Herzens in der Zeiteinheit eine bestimmte Funktion des Sauerstoffdruckes oder — wenn wir die Cyannatriumversuche mit berücksichtigen — der Geschwindigkeit der Oxydationen ist. Da die Geschwindigkeit der Herzschläge durch das Remaksche Ganglion bestimmt ist, so gewinnt man den Eindruck, als ob die im Ganglion vorhandene oder die dasselbe umspülende Masse eines bestimmten Oxydationsproduktes in jedem Zeitpunkt die Zahl der Herzschläge in der Zeiteinheit bestimmt.

Die Beeinflussung des Herzmuskels durch Sauerstoffmangel ist hier nicht berücksichtigt. Dieselbe würde nicht in erster Linie die Zahl der Herzschläge in der Zeiteinheit beeinflussen, sondern die Energie der Contractionen. Es mag nebenbei bemerkt werden, daß wir die Tendenz zur Bildung Lucianischer Gruppen bei den Versuchen an Fundulus relativ selten bemerkt haben und nur dann, wenn die Zahl der Herzschläge in der Zeiteinheit sehr gering war.

VII. Zusammenfassung der Resultate.

1. Es wird der Nachweis geführt, daß eine bestimmte Herabsetzung des Sauerstoffdruckes die Zahl der Herzschläge des Fundusembryos in der Zeiteinheit auf $^1/_2$ bis $^1/_3$ herabsetzt und daß das Herz eine Reihe von Tagen (bis zu zehn in unseren Versuchen) mit dieser Geschwindigkeit weiter schlagen kann.

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2. Wir sind noch nicht in der Lage, die Grenzwerte des Sauerstoffdruckes für diese Wirkung genau anzugeben; wir haben diese Wirkungen aber beobachtet, wenn die Luft durch ein Gemisch von Wasserstoff und Luft ersetzt war, wobei das Partialvolumen der Luft ein Viertel bis ein Zehntel des Gesamtvolumens der Mischung betrug.

3. Versuche mit NaCN haben ergeben, daß bei Zusatz geringer Mengen dieses Stoffes zum Seewasser die Herzen von Fundulusembryonen tagelang mit einer verminderten Geschwindigkeit schlagen, und daß diese Geschwindigkeit eine Funktion der Konzentration des NaCN in der Lösung ist.

4. Es wird ein Versuch gemacht, diese Tatsache unter der Voraussetzung zu erklären, daß die Zahl der Herzschläge durch die Masse der Konzentrationen eines Stoffes bestimmt wird, der vornehmlich durch Oxydation gebildet wird, der in beschränktem Maße, aber auch durch Hydrolyse entstehen kann.

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(From the Department of Physiology and Pharmacology of the Rockefeller Institute.)

Morphin und Kardioektomie.

Von **T. S. Githens** und **S. J. Meltzer.**

(Der Redaktion zugegangen am 18. März 1912.)

I.

Morphineinspritzungen nach der Kardioektomie.

Vor einiger Zeit wurde in diesem Zentralblatt[1]) von einem von uns (Meltzer) die merkwürdige Tatsache mitgeteilt, daß bei entherzten Fröschen eine Einspritzung von 6 bis 8 mg Morphin einen Tetanus innerhalb 30 bis 40 Minuten hervorbringt, während doch bei intakten Fröschen ein solcher Erfolg entweder niemals oder erst nach langer Zeit eintritt. Später wurde jedoch ergänzend berichtet[2]), daß mit dem Einsetzen des Frühlings die krampferzeugende Wirkung des Morphins bei den entherzten Fröschen zu verschwinden begann, so daß im Juni dieselbe fast gar nicht mehr erkennbar war. Dabei wurde bemerkt, daß im letzterwähnten Monat die Frösche kaum mehr als 35 Minuten die Kardioektomie überlebten. Mit dem Einsetzen der kälteren Jahreszeit haben wir das Studium der bezüglichen Verhältnisse von neuem aufgenommen und wollen wir hier einige Ergebnisse desselben kurz mitteilen.

Zunächst wollen wir den allgemeinen Einfluß der Kälte mit ein paar Worten erwähnen. Der Einfluß der Temperatur auf die Lebensdauer der entherzten Tiere war schlagend. An warmen Tagen im Dezember, bei etwa 21° C Zimmertemperatur, betrug die Lebensdauer etwa 55 Minuten; dagegen schwankte die Lebensdauer im Januar, bei einer Temperatur von 5° C (am offenen Fenster), zwischen 5 bis 7 Stunden. Ferner konnten wir den begünstigenden Einfluß der Kälte auf die erregende Wirkung des Morphins auch bei intakten Fröschen deutlich konstatieren. So konnten wir bei intakten Fröschen, welche im Refrigerator aufbewahrt wurden, bereits 7 Stunden nach einer Einspritzung von nur 0·1 mg per Gramm Frosch das Auftreten eines unzweideutigen Tetanus beobachten, der aber nachließ, sobald das Tier

[1]) XXV, 49, 1911.
[2]) Dies Zentralbl., XXV, 484, 1911.

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ins warme Zimmer gebracht wurde, um mit der Rückkehr nach dem Refrigerator wieder einzusetzen.

Was den Einfluß auf die kardioektomierten Frösche anbetrifft, so wurde in dieser Versuchsreihe das Morphin per Gramm Frosch dosiert. Die Herabsetzung der Temperatur wurde besorgt an kalten Tagen durch das Halten der Tiere am offenen Fenster und an warmen Tagen durch Aufbewahren der Frösche in Kältegefäßen und ähnlichen Einrichtungen. Die Resultate bestätigen zunächst im allgemeinen den zuerst aufgestellten Satz, nämlich daß die Kardioektomie die konvulsierende Wirkung des Morphins sehr fördert. In den sehr vielen Versuchen waren nur sehr wenige Ausnahmen, in denen kein ausgesprochener Tetanus auf die Morphineinspritzung folgte, und diese Ausnahmen fanden meistens an warmen Tagen statt. Der begünstigende Einfluß der Kälte war hier äußerst schlagend. Bei einer Zimmertemperatur von 13 bis 14° C konnte schon eine Dose von nur 0·03 mg Morphin per Gramm Frosch einen ausgesprochenen Tetanus innerhalb einer Stunde hervorbringen, was für einen Frosch von 30 Gramm eine Dose von weniger als einen Milligramm bedeutet! In vereinzelten Fällen vermochten sogar noch kleinere Dosen einen Tetanus herbeizuführen. Nach größeren Dosen waren die tetanischen Anfälle viel heftiger und namentlich von längerer Dauer. In dieser Versuchsreihe kamen die Depressionserscheinungen seltener zum Vorschein als in der ersten Versuchsreihe. Die Kälte scheint eher das Auftreten der Erregung als das der Depression zu fördern. In den meisten Versuchen wurde die Morphineinspritzung fast konstant 6 bis 10 Minuten nach der Kardioektomie vorgenommen. Die Länge des Intervalls zwischen der Einspritzung und dem Auftreten des Tetanus schwankte zwischen 40 und 70 Minuten, meistens abhängig von der Größe der eingespritzten Dose. Es stellte sich aber später heraus, daß der Tetanus viel eher nach der Einspritzung eintrat, wenn das Intervall zwischen Kardioektomie und Einspritzung länger war, so daß in einzelnen Fällen der Tetanus bereits 3 respektive 5 Minuten nach der Einspritzung auftrat, nachdem letztere erst 55 Minuten nach der Kardioektomie erfolgte. Auf die Einzelheiten dieser Vorkommnisse und deren mögliche Bedeutung sind wir noch nicht bereit einzugehen. Nur wollen wir nicht verfehlen, darauf hinzuweisen, daß offenbar auch ohne die kardiale Zirkulation eine subkutane Injektion einen allgemeinen funktionellen Erfolg in sehr kurzer Zeit zu bewirken imstande ist.

II.

Kardioektomie nach Morphineinspritzung.

Spritzt man gleichzeitig mehreren Fröschen eine passende Dose von Morphin ein und exzidiert später bei einem Teile dieser Tiere das Herz, so fördert das Experiment ein schlagendes Resultat zutage: während die intakten Tiere normal bleiben, stellt sich bei den kardioektomierten Tieren ein mehr oder weniger lange währender, heftiger

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Tetanus ein. Wir haben eine längere Reihe solcher Versuche ausgeführt. Auch bei diesen Versuchen zeigte es sich, daß die Kälte auf den Eintritt der Konvulsionen fördernd wirkt; der Einfluß war aber lange nicht so einschneidend wie bei den Tieren, bei denen Morphin nach der Entherzung appliziert wurde. So ist es oft vorgekommen, daß bei derselben Dose und zur selben Zeit und ganz unter denselben Bedingungen die Frösche, bei denen die Kardioektomie der Morphineinspritzung voranging, ohne tetanische Erscheinungen zugrunde gingen, während die Tiere, welche ihr Morphin vor der Kardioektomie erhielten, in einen heftigen Tetanus verfielen. Auch bei der Reihenfolge Morphin-Kardioektomie kam es vor, daß in der Kälte schon eine Dose von nur 0·01 mg Morphin per Gramm Frosch, also für mittelgroße Frösche nur ein kleiner Bruchteil eines Milligramms für das ganze Tier, genügte, einen heftigen Tetanus hervorzurufen. Die kleinste Dose, welche wir jedoch in einer größeren Zahl von Experimenten ausprobiert haben, betrug 0·03 mg per Gramm Frosch. Bei dieser Dose sind wir einer größeren Zahl von Fehlschlägen begegnet; doch waren die Fehlschläge in der Minderzahl und ereigneten sich meistens an warmen Tagen. Mit der Erhöhung der Dose verschwanden die negativen Resultate fast vollkommen, um sich bei Dosen von 0·3 bis 0·5 mg per Gramm Frosch wieder einzustellen, und hier auch nur, wenn das Intervall zwischen der Morphineinspritzung und der Kardioektomie nicht viel länger als eine halbe Stunde betrug. Größere Dosen als 0·5 g per Gramm Frosch haben wir in dieser Versuchsreihe nicht untersucht. Die Länge des Intervalls zwischen der Exzision des Herzens und dem Eintritt des Tetanus variierte im allgemeinen zwischen 30 und 90 Minuten, mit vereinzelten Ausnahmen nach oben wie nach unten. Genaue, gesetzmäßige Beziehungen konnten nicht festgestellt werden. Doch befanden sich die längeren Intervalle meistens bei den kleinen Dosen und die kürzeren Intervalle bei den größeren Dosen des Morphins und namentlich bei den gleichzeitig längeren Intervallen zwischen der Einspritzung und der darauf folgenden Kardioektomie. In den meisten Fällen ging dem Tetanus eine Periode voraus, in welcher das Tier deutlich hyperästhetisch und spastisch war. Diese Periode fehlte überhaupt nur selten, auch in den Fällen, in denen ein ausgesprochener Tetanus völlig ausblieb. Der Tetanus war in den meisten Fällen heftig und konnte von einem Strychnintetanus kaum unterschieden werden. Die Dauer des Tetanus variierte zwischen wenigen Minuten und mehreren Stunden; in den meisten Fällen jedoch hielt der Tetanus mindestens eine Stunde an. In dieser Versuchsreihe ging der Hyperästhesie in nur wenigen Fällen eine ausgesprochene Depression und Parese des Tieres voran. Die Lebensdauer der morphinisierten Tiere nach der Kardioektomie war stets bedeutend länger, wenn sie tetanisch waren, als wenn sie ohne tetanische Erscheinungen zugrunde gingen. Kardioektomierte, aber nicht morphinisierte Tiere lebten jedoch länger als Tiere, welche vor der

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Kardioektomie Morphin erhielten. Dieser Unterschied kam namentlich bei niedriger Temperatur deutlich zum Vorschein.

In dieser Versuchsreihe wurde das Gift, wenigstens ein gut Teil desselben, dem Zentralorgane durch die Zirkulation zugeführt, namentlich in den Fällen, in denen das Intervall zwischen der Morphineinspritzung und der Herzexzision nicht zu kurz war. Nach Ausschaltung der Zirkulation stellten sich nach verhältnismäßig kurzer Zeit strychninartige Krämpfe ein. Die Fortdauer der Zirkulation muß demnach etwas enthalten, das den Ausbruch der Krämpfe verhindert.

Die Untersuchungen wurden in weitaus den meisten Fällen an Rana pipiens ausgeführt und nur in wenigen Fällen wurden Versuche auch an Rana clamitans angestellt. Bei der prinzipiellen Bedeutung, welche den Ergebnissen dieser Versuche zukommt, wäre es wünschenswert, daß die Resultate an europäischen Fröschen nachgeprüft werden.

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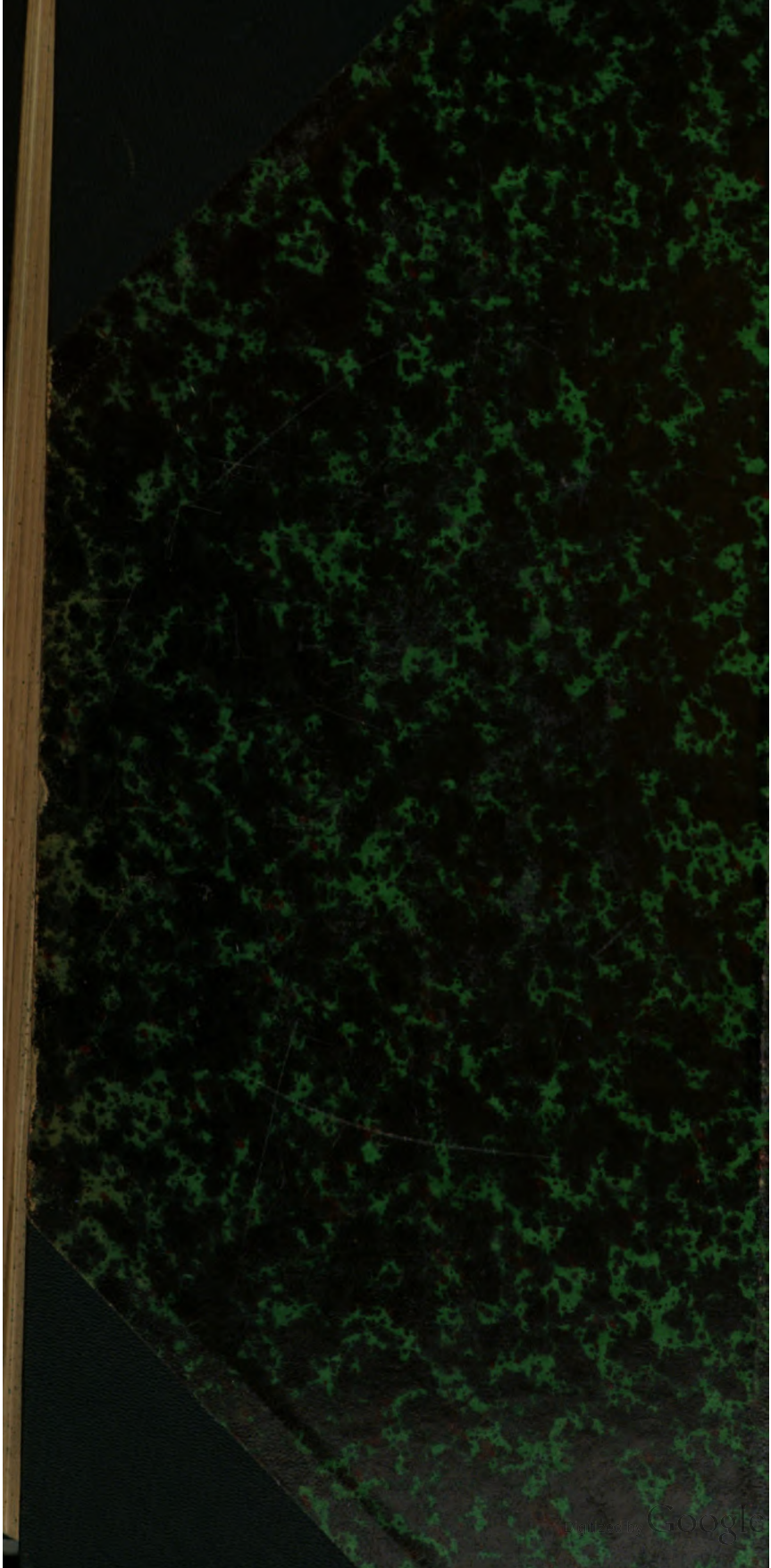
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